Development and Regeneration of the Pineal Region of the Diencephalon

Thesis presented for the degree of MPhil

by Jiahui Liu

Supervisor Prof. Claudio Stern

Department of Cell and Developmental Biology

University College London

September 2013

I, Jiahui Liu, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Organizer is a group of cells that induces and patterns surrounding tissues during embryo development. Previous studies of organizers were mainly based on transplantation of various pieces of tissues. This project first aimed to find out putative organizers using a novel method, which was to characterize organizers based on patterns of syn-expression genes. The differential microarray assays selected a list of gene that are enriched or depleted in three known organizers (Hensen's node, notochord and floor plate and zone of polarizing activity). Whole-mount *in-situ* hybridisation, histological sections and optical projection tomography were used to further analyse the expression patterns of these syn-expressed genes. The roof of dorsal thalamus including the pineal gland is revealed as one of a potential organizer, with expression of *cNOT1, TSPAN6* and *PKIγ*.

Further studies of gene expression showed that *cNOT1* is expressed broadly in early diencephalon and is restricted to pineal gland and its posterior territory till HH25. This raises questions that whether *cNOT1* is marking the pineal progenitors and whether there are movements of *cNOT1*-expressing cells. Fate mapping analysis demonstrates that not all *cNOT1*-expressing cells are pineal progenitors and they are not moving over the development of pineal gland.

Considering the change of gene expression over diencephalon development, it could be that either *cNOT1*-expressing cells or the pineal gland is a potential organizer. To assess their organizing abilities, ablation experiments were performed on both area. Ablation of pineal progenitors alone led to regeneration of the whole pineal gland and with normal expression patterns of diencephalic genes. On the other hand, removal of all *cNOT1*-expressing cells inhibited the regeneration of pineal gland.

In conclusion, these data suggest that the pineal gland can be regenerated from surrounding tissues. Whether the roof of the dorsal thalamus or the *cNOT1*-expressing cells is an organizer requires more experiments, including transplantation, to assess.

Table of Contents

Chapter 1
Introduction

1 Introduction

Organizer is a group of cells that induces and patterns surrounding tissues during the development of embryos. It was first demonstrated by Hans Spemann and Hilde Mangold in 1924 and is an important landmark in the history of developmental biology (Spemann and Mangold, 2001; Spemann, 1924b). Organizers equivalent to the Spemann's organizer were also discovered in other animals including mouse, *Xenopus*, zebrafish and chick (De Robertis et al., 2000; Niehrs, 2004; Shih and Fraser, 1996; Waddington, 1933), which shows that it is conserved between species.

Different to signalling centres, organizers can not only provide signals to surrounding cells, but also drive cellular rearrangements nearby. Transplantation of organizers to ectopic sites can alter cell fates of surrounding tissues, while ablation of organizer will cause abnormal development. Based on these criteria, there are only five organizers found in chick embryos, which are Hensen's node (equivalent to the Spemann's organizer), notochord and floor plate, zone of polarizing activity (ZPA), isthmus and apical ectodermal ridge (AER). How these organizers were discovered and function in embryos will be discussed in the chapter of introduction in detail, following the order of development.

1.1 Hensen's node

The first organizer that appears during chick embryo development is Hensen's node. At the end of gastrulation, Hensen's node is formed at the anterior end of the primitive streak, which is an organizer in chick embryo. Hensen's node is equivalent to Spemann's organizer (also called "the organizer"), which is the first organizer discovered by Spemann and Mangold. In 1907, Warren Lewis transplanted the dorsal lip of a blastopore at gastrula stage to the ventral ectopic side of a similar stage embryo and observed the formation of a secondary axis (Lewis, 1907). But he concluded it to be the result of self-differentiation of the graft. Until 1924, when Spemann and Mangold figured out a way to distinguish

host and donor tissues and repeated the experiment successfully to show that the donor dorsal lip could induce and pattern the host tissues to form a secondary axis (Spemann, 1924b). They exchanged pieces of tissues between Triton embryos of different species and analysed the cell fates of those transplanted embryos. Triton Cristatus cells are unpigmented and can be easily distinguished from pigmented *taeniatus* or *alpestris* cells over a long time. They have observed that most tissues were influenced by surrounding tissues and differentiated according to their new positions. However, there is one exception that acted differently, which is the upper lip of the blastopore. It induced a formation of secondary axis containing neural tube and somites in the region of epidermis progenitors (Spemann, 1918; Spemann, 1924b). Importantly, the neural tube was derived almost entirely from host tissue, whereas the graft gave rise mainly to the notochord. Therefore Spemann defined an organizer as a piece of tissue that can induce a secondary axis when it is transplanted to an undifferentiated region of another embryo (Spemann, 1924a; Spemann, 1924b). The primary (gastrula) organizer is conserved across different species including *Fundulus*, zebrafish, *Xenopus*, chick, quail, duck, rabbit and mouse, which were also proven by transplantation experiments (Beddington, 1994; Oppenheimer, 1936b; Shih and Fraser, 1996; Spemann, 1924a; Waddington, 1932a; Waddington, 1933). In *Fundulus* and zebrafish, microsurgical grafting of the embryonic shield to an ectopic side can induce a secondary axis containing both graft and host cells, which demonstrates that the embryonic shield is equivalent to the Spemann's organizer (Ho and Kimmel, 1993; Oppenheimer, 1936a; Oppenheimer, 1936b). On the other hand, Waddington performed transplantation experiments between rabbit, chick and quail embryos and thus identified Hensen's node, which is the tip of primitive streak, as the equivalent of "Spemann's organizer" (Hensen, 1875; Storey et al., 1995; Waddington, 1932b; Waddington, 1933; Waddington, 1936). Similarly, the anterior part of the primitive streak in the mouse resembles the chick Hensen's node. Transplantation of the mouse node can induce additional digit in chick limb buds and anterior structures in *Xenopus* (Blum et al., 1992b; Hogan et al., 1992; Kintner and Dodd, 1991). Later on, Beddington showed that grafts of mouse node posterolateral location induced a secondary axis and the formation of ectopic somites, which confirmed that the mouse node is the equivalent of Spemann's organizer (Beddington, 1994). In addition, cross-species transplantations between chick, zebrafish, rabbit, amphibians and mouse proved

that the properties and functions of organizers are conserved (Blum et al., 1992a; Hatta and Takahashi, 1996; Kintner and Dodd, 1991; Oppenheimer, 1936a; Waddington, 1934; Waddington, 1936; Waddington, 1937)

Although Waddington showed that transplantation of Hensen's node to an ectopic lateral site in duck, rabbit or chick embryo could all induce a secondary axis as Spemann's organizer (Waddington, 1932a; Waddington, 1933), this ectopic site was later shown to develop into neural plate and thus it was not possible to conclude that the host cells change their fate (Stern, 2005). The organizing ability of Hensen's node was later proved by the transplantation in ectopic site at area opaca (Figure1.1), which only develops into extraembryonic structures (Dias and Schoenwolf, 1990; Guttikar et al., 1993; Storey et al., 1992). The competence of this region will no longer exist after HH5 (Dias and Schoenwolf, 1990; Gallera, 1970; Gallera and Ivanov, 1964; Storey et al., 1992; Streit and Stern, 1997). After ablation of the organizer (under certain conditions), embryos can still develop normally, which shows that the organizer may be regulated by the surrounding tissues as well (Abercrombie and Bellairs, 1954; Butros, 1967; Waddington, 1932a). More recent investigations suggest that the organizer is a dynamic state rather than a pre-established population of cells, and that this state is determined by signals emanating from a "node inducing centre" located in the middle of the primitive streak and expressing *Vg1* and *Wnt8c*, which induce adjacent cells to become organizer (Joubin & Stern 1999). This explains the finding that as cells move in and out of Hensen's node they acquire and lose node markers appropriate to their current position, demonstrated by DiI labelling experiments coupled with hybridisation of the labelled embryos (Joubin and Stern, 1999). The role of *Vg1* and *Wnt8C* in defining the organizer state in this experiment has a parallel in the events of much earlier development, prior to primitive streak formation, where *Vg1* and *Wnt8C* cooperate to induce *Nodal*, and through this a primitive streak containing the future organizer at its tip (see above and Skromne & Stern 2001; 2002; Bachvarova et al., 1998). Bachvarova et al. found that the posterior marginal zone (PMZ) (where *Vg1* and *Wnt8C* expression overlaps) can induce primitive streak and the Hensen's node without contributing cells to these structures (Bachvarova et al., 1998). In this respect (the ability to induce an organizer without making a cellular

contribution to it), the PMZ fulfils the requirements to be equivalent to the Nieuwkoop centre of Amphibians. Nieuwkoop centre induces the neighbouring Spemann's organizer by dorsalizing Nieuwkoop signals (Nieuwkoop, 1992). In addition, mesoderm induction assays in *Xenopus* and mouse models confirm the induction of the organizer by *Vg1* and *Wnt* signalling pathway is conserved across species (Watabe et al., 1995).

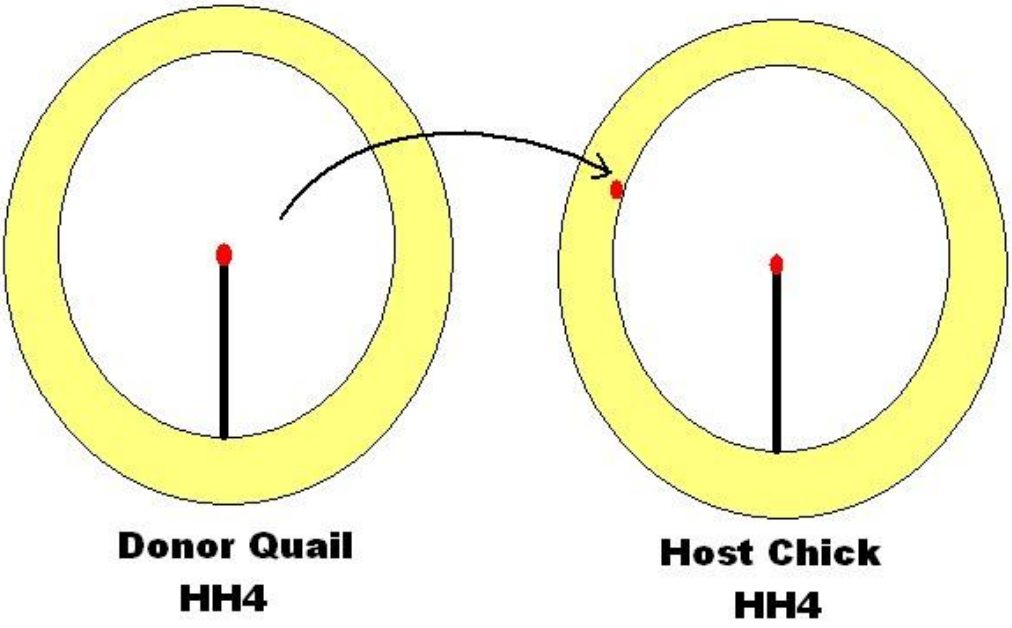


Figure 1.1 Transplantation of Hensen's node. Hensen's node (red) is transplanted from donor quail embryo at HH4 to area opaca (yellow area) at host chick at HH4. After incubation, a secondary axis will be developed in the host ectopic side.

Following the discovery of the organizer, many studies have been concentrated on its functions and the molecular mechanism of its action. The first gene claimed to be specific for the organizer was *goosecoid* isolated in 1991 (Cho et al., 1991). Apart from its expression in the dorsal lip of the blastopore, microinjection of *goosecoid* mRNA at an ectopic site leads to the formation of secondary axis in *Xenopus* (Cho et al., 1991). *Goosecoid* expression is induced by activin (which belongs to the same class of TGFβ-related molecules as *Vg1* and *Nodal*) (Cho et al.,

1991). Gene expression and fate mapping studies in chick embryo also show that *goosecoid* is a marker for organizer cells (Izpisua-Belmonte et al., 1993). However it is also expressed in non-organizer tissues including Koller's sickle, prechordal mesoderm and head endoderm (Izpisua-Belmonte et al., 1993). In the mouse, initial studies using *gsc*-null mutants showed that *goosecoid* is not required for formation of the primary axis (Rivera-Perez et al., 1995). However, an effect on neural inducing properties was revealed by a more sensitive assay based on cross-species transplantation of the mouse node to chick embryos, which showed that *goosecoid* affects the neural inducing strength of the node (Zhu et al., 1999a).

Later, mainly by using differential screens of cDNA libraries, many more genes expressed in the amphibian organizer were discovered including *frzb-1*, *cerberus*, *chordin*, *noggin*, *follistatin* and *dickkopf -1* (*DKK-1*) (Bouwmeester et al., 1996; Fainsod et al., 1997; Glinka et al., 1998; Hemmatibrivanlou and Melton, 1994; Leyns et al., 1997; Piccolo et al., 1996; Sasai et al., 1996). Among these molecules, chordin, noggin and follistatin can induce neural tissues in a *Xenopus* animal cap assay (Hemmatibrivanlou et al., 1994; Lamb et al., 1993; Piccolo et al., 1996; Sasai et al., 1996). Microinjection of mRNA encoding any of these three proteins can also mimic the effect of the organizer and cause the formation of secondary neural tube, dorsal mesoderm and additional gut (De Robertis et al., 2001; Hemmatibrivanlou and Melton, 1994; Holley et al., 1995; Lamb et al., 1993; Schmidt et al., 1995). Further studies revealed that both chordin, noggin, follistatin, and other neural inducing molecules are BMP inhibitors (Hansen et al., 1997; Piccolo et al., 1999; Piccolo et al., 1996; Zimmerman et al., 1996). This, along with other findings (Hemmati-Brivanlou and Melton, 1997; Stern, 2005), led to the establishment of the "default model" of neural induction, which proposes that ectodermal cells will develop into neural tissues if they do not receive BMP signals, otherwise they are instructed to develop into epidermis (Wilson and Hemmatibrivanlou, 1995). According to this model, the organizer would act by lowering the BMP concentration around ectoderm cells and thus preventing them from being induced to epidermis by BMP. BMP signalling is also particularly important for patterning mesoderm (Dosch et al., 1997; Tonegawa et al., 1997; Winnier et al., 1995).

However, it was first noticed in chick embryos that the expression patterns of BMPs and their antagonists did not fit the "default model" (Streit et al., 1998). Moreover, misexpression of *chordin* in ectopic regions does not induce the expression of early neural markers (Linker and Stern, 2004; Streit et al., 2000; Streit et al., 1998). Experiments in *Xenopus* also show that neural induction of *chordin* or *noggin* is inhibited by blockage of FGF signalling pathway (Launay et al., 1996; Linker et al., 2009; Linker and Stern, 2004; Sasai et al., 1996). Therefore, inhibition of BMP is not sufficient or requires cooperation of other signalling molecules to induce neural development in chick.

Apart from dorsalization of the mesoderm and neural induction, the organizer also plays a role in orchestrating left-right asymmetry at the late primitive streak stage in avian and mammalian embryos. The first insights into this process came from the observations that mRNA encoding the "activin" (actually Nodal) receptor cActRIIA is expressed on the right side of the chick primitive streak at stage HH4 (Levin et al., 1995; Stern et al., 1995), while the transcription factor HNF3β (Foxa2) and the secreted protein Sonic hedgehog are expressed on the left side of the node (from HH4+ to HH6) (Levin et al., 1995). Soon afterwards (HH6-7), *nodal* expression starts as a small spot next to the left node and a larger domain in the left lateral plate. Misexpression and knockdown of these components affect these expression patterns and reveal a pathway that initiates left-right asymmetry (Levin, 1997; Levin et al., 1995; Levin et al., 1997; Tabin, 2006). Expression of *nodal* was shown to be induced by Shh (Levin et al., 1995), while misexpression of *activin* can repress *SHH* expression on the left hand side and induce the expression of right-hand side markers (Levin, 1997; Levin et al., 1995). Also, misexpression of *nodal*, *SHH* or *activin* can affect heart situs (Levin et al., 1997; Zhu et al., 1999b). The cascade of signals converges on the transcription factor Pitx2, which shows left-sided expression in all organ systems that develop asymmetrically (Campione et al., 1999; Capdevila et al., 2000; Yokouchi et al., 1999; Yoshioka et al., 1998; Zhu et al., 1999b). Surprisingly, of these various asymmetry markers, only two (Nodal and Pitx2) are conserved across all vertebrates (Collignon et al., 1996; Levin et al., 1995; Long et al., 2003). The mechanism that position these two seems quite variable in different species, and has been shown to involve diverse strategies such

as ion fluxes (Levin, 2003), ciliary beating (Babu and Roy, 2013; Nonaka et al., 1998; Okada et al., 1999), dynein (Supp et al., 1997), calcium signalling (Langenbacher and Chen, 2008) and other signals. For example, *nodal* expression in mouse and zebrafish is not as essential as that in chick and *Xenopus*. In mouse chimera embryos, no defects have been observed in left-right asymmetry (Beddington and Robertson, 1999). Similarly, point mutations in *nodal* homologous *cyclops* (*cyc*) in zebrafish does not affect heart looping as well (Chen et al., 1997). In fact, the breakthrough of symmetry in mouse is affected by the *nodal* flow generated, which is a leftward flow of extracellular fluid generated by monocillia around the node and requires the axonemal dynein, left-right dynein (lrd) (McGrath et al., 2003). Similar nodal flow mechanism is also observed in zebrafish, and knockdown of *lrd* causes randomise gene expression and cardiac laterality (Essner et al., 2005; Kawakami et al., 2005).

In conclusion, the Spemann's organizer and its equivalent in other species are responsible for the development of primary anterior-posterior and left-right axis. Genes like *SHH* and *chordin* are involved within the process and conserved across different species. But variations in mechanisms of organizing activities are also present.

1.2 Notochord and floor plate

Following Spemann's organizer, the second organizer involved in embryo development is the notochord and floor plate, which is responsible for the dorsal-ventral patterning of neural tube.

Following the regression of the primitive streak and the formation of neural plate, the neural tube starts to form by bending of the neural plate into a hollow tubular structure. DiI labelling reveals that the anterior-median quadrant of the epiblast of Hensen's node contributes to cells in both the notochord and the ventral-most wedge of the neural tube, which is the prospective floor plate (Selleck and Stern, 1991). This can be seen even when a single cell in this node region is labelled with a lineage tracer: descendants can be found in both the notochord (mesoderm) and

ventral neural tube (ectoderm) (Selleck and Stern, 1991). This pattern persists at later stages of development; quail-chick grafting experiments show that the floor plate of the neural tube and the notochord both derive from same group of cells from the midline of Hensen's node at stages 6-8 (Catala et al., 1996). In addition, ablation of the notochord prevents the formation of the floor plate if done early enough (Artinger and Bronnerfraser, 1993; Placzek et al., 1991; van Straaten and Hekking, 1991). Conversely, transplantation of notochord to an ectopic site next to the lateral neural tube demonstrates that the notochord can induce the formation of an additional floor plate (Figure1.2) and the expression of HNF3β, which is expressed transiently in the notochord and later in the floor plate during development (Altaba et al., 1995; Placzek et al., 1990; Vanstraaten et al., 1985). The notochord induces not only a floor plate, but also motor neurons in the ventral neural tube (Placzek et al., 1990; Placzek et al., 1991; Vanstraaten et al., 1985).

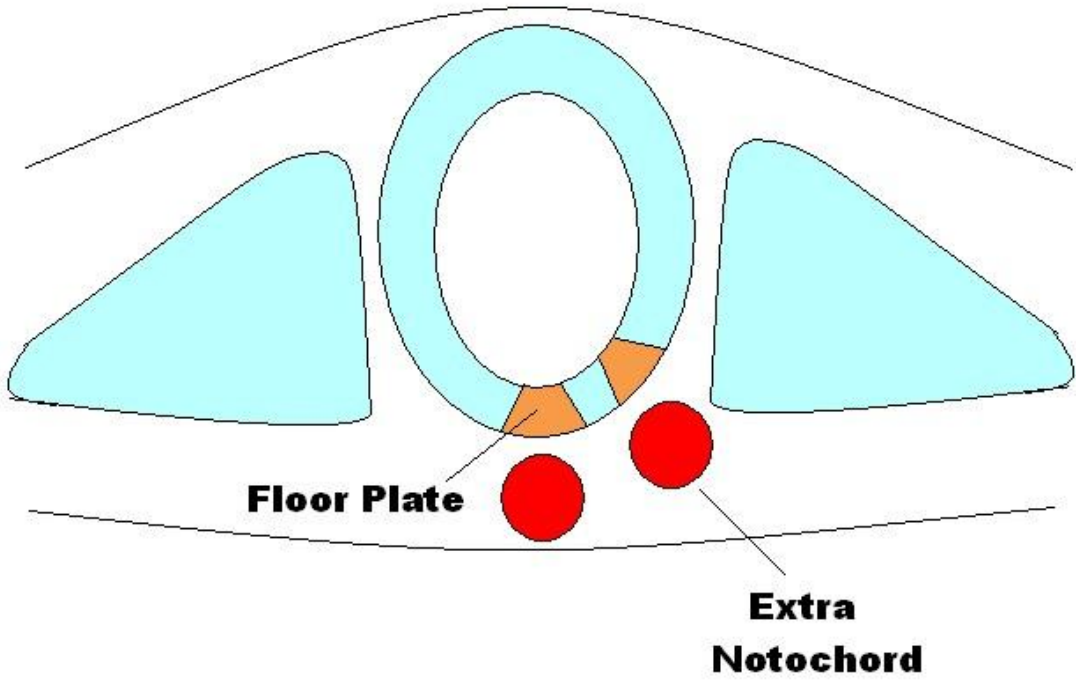


Figure 1.2 Transplantation of notochord can induced an extra floor plate in chick embryo.

However, some have questioned the necessity of notochord for the induction of floor plate and motor neurons. One evidence is zebrafish mutants *floating head (flh)* and *no tail (ntl)* mutation embryos lack a notochord but still have a normal floor

plate (Halpern et al., 1993; Halpern et al., 1995). It was also argued that the ablation experiments in chick may have included the removal of predetermined floor plate precursors (Catala et al., 1996).

Despite this dissent, the inductive ability of the notochord has been reproduced many times and is now understood to be based on its secretion of Shh, which is first expressed in the notochord and then in the floor plate (Echelard et al., 1993; Krauss et al., 1993; Roelink et al., 1994). Misxpression of *SHH* in mouse, frog or zebrafish embryos mimics the effect of notochord transplantation and induces the formation of extra floor plate, motor neurons and ventral interneurons (Echelard et al., 1993; Krauss et al., 1993; Roelink et al., 1994). In support of this idea, further analysis in chick embryos provides more evidence for the inducing ability of the notochord and Shh: first, time-lapse microscopy revealed that many floor plate cells derive from epiblasts anterior to Hensen's node (named "area a") and therefore do not share a lineage with the notochord cells. Second, co-expression of *nodal* and *SHH* induces the formation of floor plate from "area a" cells *in vitro* (Patten et al., 2003). In conclusion, the notochord induces the formation of floor plate and establishes ventral identities in the neighbouring neural tube. Apart from the notochord, the floor plate itself also plays a role in patterning the neural tube through secretion of diffusible signals including Shh, Wnt and BMP (Dale et al., 1999; Furuta et al., 1997; Liu et al., 2000; Mcgrew et al., 1992; Placzek et al., 1993; Placzek et al., 1991; Yamada et al., 1993). This implicates a relay system: the notochord provides the initial Shh signal, which induces the floor plate, and the floor plate itself secretes Shh responsible for inducing motor neurons and ventral interneurons in the adjacent ventral neural tube. It is now widely believed that Shh acts as a gradient, highest ventrally and diminishing dorsally, where it is also antagonised by BMP and other TGFβ molecules expressed by the roof of the neural tube (Liem et al., 2000; Liem et al., 1997; Liem et al., 1995). Based on the concentration of Shh, different transcription factors and downstream genes are activated or repressed. As a result, different types of neurons are generated across the neural tube (Dessaud et al., 2008; Liem et al., 2000). For example, *Pax6* is repressed by Shh and is expressed more dorsally in the neural tube (Liem et al., 2000). On the other hand, *Nkx2.2* is activated by Shh and is expressed in the ventral part of the neural tube (Liem et al., 2000). This mechanism generates the diversity of neuronal territories

along the dorso-ventral axis of the spinal cord (Briscoe et al., 2001; Briscoe and Ericson, 2001; Briscoe et al., 2000; Jessell, 2000).

1.3 Organizers involved in brain development

As a complex organ, there are two organizers found in brain development, which are the anterior neural ridge (ANR) and isthmus. These two organizers are responsible for patterning different compartments in brain vesicles. The ANR directs the development of the forebrain, while the isthmus is responsible for the development of the mesencephalon and the metencephalon. The following chapter will discuss their discovery and functions in brain development separately. I will also introduce ZLI, which is considered to be an organizer in diencephalon in many research paper.

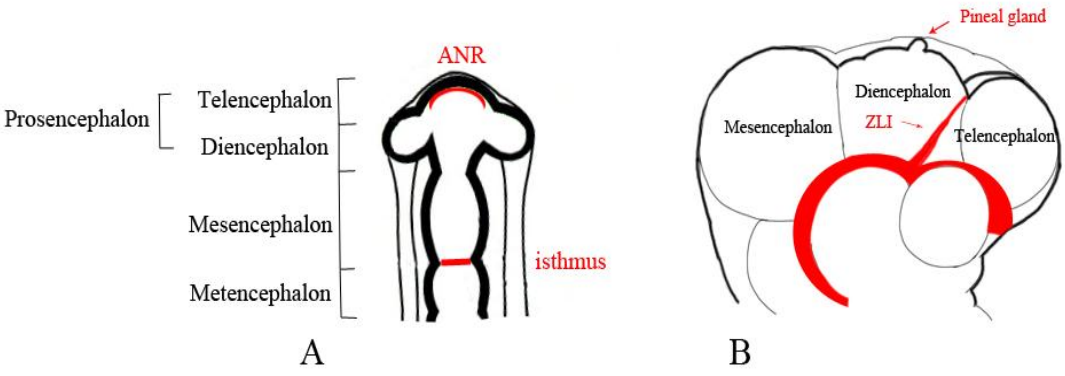


Figure 1.3 Structures of brain vesicles in chick embryo. A) primary brain vesicles at HH 10; B) brain compartments at HH22. Red area shows the expression of *SHH*.

1.3.1 Anterior neural ridge

During the development of the notochord in chick embryo, the head fold starts to form at the anterior neural plate. First, all three layers (ectoderm, mesoderm and endoderm) fold ventrally, forming an arc that progress caudally; this is called the head fold in chick and mammalian embryos. The endodermal lining of this head fold first defines the foregut which gradually becomes a tube (Bellairs, 1954;

Kimura et al., 2006). As the neural plate folds to form a tube, this process continues in the head, involving the medial neural plate in the dorsal part of the head fold. From stages HH9-HH10 (Figure1.3 A), bulges appear in this region, which will give rise to the so-called primary brain vesicles: prosencephalon (forebrain), mesencephalon (midbrain) and rhombencephalon (hindbrain, containing metencephalon). The prosencephalon is further subdivided into telencephalon and diencephalon (Hamburger and Hamilton, 1951; Hamburger and Hamilton, 1992). At stage HH8, the forming anterior neural plate of the chick displays a pair of prominent protrusions, which will eventually form the olfactory placode. The anterior edge of the neural plate, which is also called the anterior neural ridge (ANR) (Figure1.3 A), is suggested to act as an organizer that regulates the development of the telencephalon. In the mouse, a mutation of *BF1* (now called *FOXG1*) causes a reduction in size of the cerebral hemispheres in the telencephalon due to the reduction of proliferation of neuroepithelial cells (Xuan et al., 1995). *FGF8* is strongly expressed in the ANR and induces *FOXG1* expression in the neighbouring telencephalon, suggesting that the ANR may pattern the telencephalon (Shimamura and Rubenstein, 1997b). Overexpression of *FGF8* in *Xenopus* proves that it promotes the expression of anterior telencephalic marker *FOXG1* (Eagleson and Dempewolf, 2002). Increased expression of *FOXG1* is induced by the transplantation of ANR, which demonstrates the induction ability of ANR (Eagleson and Dempewolf, 2002). However, transplantation of medial ANR inhibits the telencephalic development, while lateral ANR increases it (Eagleson and Dempewolf, 2002). It means that there are inhibitory mechanisms to ensure the development of bilateral telencephali (Eagleson and Dempewolf, 2002; Hongo et al., 1999). Transplantation and ablation of anterior ectodermal cells in the central nervous system in zebrafish also point to an important signalling function of the anterior region of the forebrain (Houart et al., 1998a). This was suggested to be due to the production of a Wnt antagonist called TLC (Houart et al., 1998a), but no homologue has yet been found in other vertebrates although there is considerable evidence from many model systems that inhibition of Wnt signalling is important for specifying anterior identity in the neural plate (Belo et al., 2009; Niehrs, 2006). The *FGF8* mutant *Ace* (“acerebellar”) in zebrafish also shows that Fgf8 is required for normal development of both the cerebellum (derived from the anterior hindbrain adjacent to the isthmus/MHB, where *FGF8* is also expressed; see below) and of the

telencephalon (Shanmugalingam et al., 2000). In chick embryo, induction or inhibition of Fgf8 does not appear to affect telencephalic development, which may due to several Fgf signalling molecules present together in the developing forebrain (Gunhaga et al., 2003). Requirement of other Fgfs like Fgf3 for telencephalon development is also shown in zebrafish using antisense morpholino inhibition experiments (Shinya et al., 2001).

In addition, the Wnt signalling pathway is also involved in telencephalon patterning. One evidence is that targeted deletion of β-catenin causes defects in medial ganglionic eminence which results from disruption of the canonical Wnt pathway (Gulacsi and Anderson, 2008). Chordin and Noggin, which are BMP antagonists, are also shown to be involved in forebrain development in mutated mice (Bachiller et al., 2000). Similar effects of BMP are also observed in zebrafish (Barth et al., 1999; Nguyen et al., 1998).

Apart from Fgf and Wnt signalling, other molecules are involved in telencephalon development. Given the strong effect of Shh in spinal cord development, and the fact that it is also expressed within the brain (in structures that continue anteriorly from the floor plate: the basal plate/hypothalamus, infundibulum and zona limitans intrathalamica, ZLI) (Scholpp et al., 2007; Scholpp and Lumsden, 2010), it would not be surprising to find that Shh plays an important role in forebrain development. Explantation of Shh shows that it induces the differentiation of ventral neuronal cells that are normally in diencephalon and telencephalon in chick embryo (Ericson et al., 1995). Targeted mutation of *SHH* in mice causes early defects in midline structures including the notochord and floor plate (Chiang et al., 1996). Explant experiments using rat and mouse tissues also suggest that *SHH* can induce the development of ventral neurons in the telencephalon (Kohtz et al., 1998). However the dorsal telencephalon seems to develop in a largely Shh-independent way; mice lacking *SHH* develop the dorsal telencephalon normally (Chiang et al., 1996). On the other hand, defects in dorsal telencephalon is observed in *Gli3* mutant mice (Rash and Grove, 2007); the source of Shh responsible for this, if any, is unknown.

1.3.2 Isthmus

Apart from ANR, *FGF8* is also expressed in the isthmus, which is the mesencephalon-metencephalon boundary (MHB) (Figure 1.3 A). There is evidence suggesting that the isthmus may function as an organizer for the mesencephalon and metencephalon. Transplantation of the isthmus into the hindbrain induces ectopic cerebellum in chick embryo (Martinez et al., 1995), whereas transplantation into the diencephalon (posterior to the ZLI) induces graded expression of *En2*, which is a marker for mesencephalon (Ballycuif et al., 1992; Martinez et al., 1991; Sgaier et al., 2007). Conversely, transplantation of a diencephalic explant to the vicinity of the isthmus causes the transplanted cells to change their fate to tectum, which does not occur if the transplant is placed in a more anterior part of the mesencephalon (Nakamura and Itasaki, 1992; Nakamura et al., 1991; Nakamura et al., 1986).

As the isthmus is located between the mesencephalon and metencephalon, it is interesting to know how this boundary is defined. Previous studies have shown that it is set up by repression between *Otx2* and *Ggx2,* which are expressed in mesencephalon and metencephalon respectively (Broccoli et al., 1999; Hidalgo-Sanchez et al., 1999; Millett et al., 1999). Misexpression of *Otx2* (anterior) or *Ggx2* (posterior) domain or vice-versa in the mouse both cause the isthmus to become re-positioned (Broccoli et al., 1999; Katahira et al., 2000; Millett et al., 1999) and it is generally thought that Otx2 and Gbx2 mutually repress each other, with the isthmus/MHB arising at the boundary between their expression domains (Grapin-Botton et al., 1999; Sato and Joyner, 2009).

As mentioned before, *Fgf8* is expressed in the isthmus as well as in the ANR. Two splice variants of Fgf8 are produced in the isthmus, which are Fgf8a and Fgf8b (Sato et al., 2001). Misexpression of *Fgf8a* does not affect the expressions of most of the isthmus specific genes except *En1* and *En2*. Overexpression causes expansion of *En1* and *En2* expression anteriorly into the diencephalon and changes the fate of diencephalic cells to mesencephalic (Muramatsu et al., 1997; Nakamura

et al., 2004; Nakamura et al., 2000). Fgf8b exerts stronger effects on brain development. Misexpression of *FgF8b* in chick embryo inhibits *Otx2* expression but induces *Gbx2* and *Irx2* to extend to the diencephalon (Sato et al., 2001). A similar effect is also observed in transgenic mice (Liu et al., 1999). Fgf8 acts through activation of the ERK (MAP-kinase) pathway (Sato and Nakamura, 2004; Suzuki-Hirano et al., 2010). In addition to Fgf8, Wnt signalling is also involved in tectum and cerebellum development. *Wnt1* knockout mice show repressions in *En1* and *En2* expression and disruptions in midbrain and metencephalon structures (Mcmahon et al., 1992). It may be required for maintenance of *Fgf8* expression (Panhuysen et al., 2004). Implants of beads and misexpression experiments in chick and mice both show that Wnt1 interacts with Lmx1b to maintain *Fgf8* expression (Adams et al., 2000; Matsunaga et al., 2002).

1.3.3 Zone limitants intrathal

Because of its expression of *SHH*, the zona limitans intrathal (ZLI), located at the border between ventral thalamus (pre-thalamus) and dorsal thalamus (thalamus) (Fog. 1.3 B), has been suggested to be a secondary organizer for the forebrain (Kiecker and Lumsden, 2004; Scholpp et al., 2006). In turn, establishment of the ZLI requires Shh shown in both fate mapping and ectopic expression of *SHH* in chick embryo (Zeltser, 2005). Expression of *SHH* is maintained in the ZLI and regulates the development of the diencephalon. In zebrafish, increasing expression of *SHH* by injection of mRNA leads to an expansion of prethalamic and thalamic gene expressions including *DLX2A*, *LHX5*, *DBX1A*, *EMX2* and *NEUROG1*, while reducing *SHH* expression by Hh signalling inhibitor or mutated Hh co-receptor smoothened, causes a reduction of expression in these genes (Scholpp et al., 2006). OEP (EGF-CFC nodal co-receptor one-eyed pinhead) mutant embryos lacking the basal plate do not appear to have defects in diencephalon (Scholpp et al., 2006). However, using microbarrier to separate the basal plate from the ZLI in chick embryos, Vieria and Martinez (2006) observed that the inhibition of *SHH* expression by microbarriers causes structural alterations, and suggested that signals from the ZLI are important for molecular regionalization of the diencephalon (Vieira and Martinez, 2006). That Shh does play a role in diencephalon

development is suggested by gain- and loss- of function experiments in chick as well (Kiecker and Lumsden, 2004). Also, in *SHH*$^{-/-}$ mice, a reduction in size of the diencephalon is observed (Ishibashi and McMahon, 2002).

In addition to Shh, Wnt signalling may also be involved in diencephalic patterning by the ZLI. Members of the Wnt family are expressed in various regions of the diencephalon, including *Wnt1*,*Wnt2b*,*Wnt3a*, *Wnt5a* and *Wnt8b*. Inhibition of *Wnt* expression by Dkk-1 bead-implantation blocks the dorsal progression of the expression of *SHH* in the ZLI, but *Wnt* expression is not required for maintenance of *SHH* expression (Martinez-Ferre et al., 2013). In addition, that the Wnt pathway regulates the expression of *Gli3* and *L-fng* in the ZLI, is also demonstrated by implantation of Dkk-soaked beads experiments in chick (Martinez-Ferre et al., 2013). The expression of some Wnt molecules are restricted by the ZLI in chick; for example, *Wnt3* which is expressed posterior to the ZLI in the dorsal thalamus (Mattes et al., 2012). *Wnt3a* or *Wnt3* induces the expression of dorsal thalamus marker Gbx2 in explants of prospective dorsal thalamus, whereas inhibition of *Wnt* induces the expression of ventral thalamic marker Dlx2 in similar explants (Braun et al., 2003).

However, despite its expression of *SHH*, there is currently no evidence showing that the ZLI can drive the rearrangement of surrounding cells. To establish this, transplantation experiments to ectopic regions, accompanied by analysis of regional markers, are essential. Therefore, in this project, ZLI is not considered to be an organizer based on the criteria mentioned at the beginning.

1.4 Zone of polarizing activity

The final organizer that I would like to introduce is zone of polarizing activity (ZPA), which is found to be involved in limb development. ZPA is a well-characterized organizer, located in the posterior limb bud mesenchyme and is responsible for patterning the anterior-posterior (radio-ulnar) axis of the limb

(Capdevila and Izpisua Belmonte, 2001). Mirror duplication of digits is induced by transplantation of ZPA cells to an ectopic anterior location (Saunders, 1948) (Figure 1.4). Several decades later, Tickle and colleagues reported that implantation of a bead soaked in retinoic acid can mimic the effect of ZPA transplantation in chick (Tickle et al., 1982), which together with the finding that posterior limb has higher retinoic levels than the anterior limb (Thaller and Eichele, 1987), immediately raised the hope that retinoic acid may be the ZPA morphogen (Slack, 1987). Inactivation of *RALDH2* by mutation, an enzyme involved in retinoic acid synthesis, can inhibit the development of the mouse limb (Niederreither et al., 1999; Niederreither et al., 2002). However later studies uncovered that *SHH* is expressed very specifically in the ZPA (Kiecker and Lumsden, 2004). Moreover, implantation of Shh beads induces similar limb duplication as ZPA transplantation (Riddle et al., 1993b). Shh seems to act in a concentration dependent manner: using alkaline phosphatise tagged Shh, Shh protein has been found a long distance from the ZPA in the chick limb bud (Yang et al., 1997). Recently, it was reported that limb bud mesenchyme cells sample their environment and transport Shh protein by means of very long, dynamic filopodia that can span a large proportion of the limb width (Sanders et al., 2013). Cholesterol modification of Shh peptide shows a disruption of formation of anterior digits, which also proves the long-range effect of Shh (Lewis et al., 2001). One of the mechanisms is by negative feedback loop involving HIP protein (Chuang and McMahon, 1999).

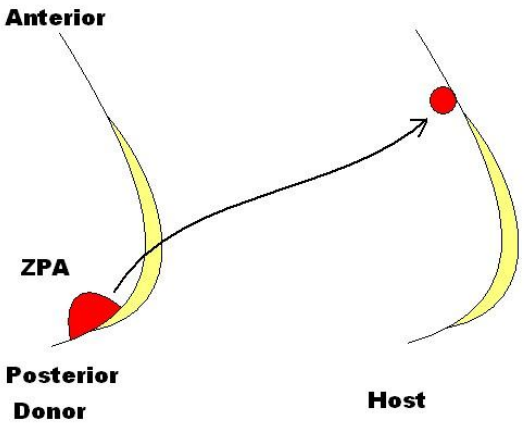


Figure 1.4 Transplantation of ZPA. ZPA located at posterior limb bud is transplanted to ectopic anterior limb bud and will induce an extra digit in the host.

Apart from the ZPA, another signalling centre has been found to act in the limb bud, the apical ectodermal ridge (AER) at the distal margin of the limb bud ectoderm; this region functions to sustain limb growth (Lu et al., 2008b). *Fgf* family members are found to be expressed in the AER including *Fgf8*, *Fgf4, Fgf9* and *Fgf17* (Lewandoski et al., 2000; Mariani et al., 2008; Niswander et al., 1993). However only Fgf8 has been shown to be necessary for limb bud development (Lewandoski et al., 2000). Inactivation of Fgf8 in AER causes a reduction of limb-bud size and delays expression of *SHH* in the ZPA, while mutations in other Fgf members do not affect limb development (Lewandoski et al., 2000). Implantation of Fgf4 beads can rescue limb development (including maintenance of *SHH* expression in the ZPA) after AER ablation (Laufer et al., 1994; Niswander et al., 1994). This reveals a positive feedback loop between the AER and ZPA to regulate limb development (Zeller et al., 2009).

1.5 Looking for putative organizers by studies of syn-expression groups

As discussed before, so far, only five organizers have been discovered that fulfil the essential criteria of "a region that has both inducing and patterning activity on surrounding cells": Hensen's node, the notochord and floor plate, the isthmus (midbrain-hindbrain boundary), the Zone of Polarizing Activity (ZPA) and the anterior neural ridge (ANR). All of these organizers were discovered based on transplantation experiments showing their inducing ability and ablation experiments that shows their requirement for normal embryological patterning. For example, Hensen's node in chick is identified as the equivalent of "Spemann's organizer" by transplantation experiments between chick and quail (Waddington, 1932a; Waddington, 1933) and ablation experiments at different developmental stages (Psychoyos and Stern, 1996).

Considering the complexity of embryo development, one would expect that there should be many more organizers remaining to be undiscovered. However the demonstration of organizer activity requires transplantation of the appropriate signalling cell to an ectopic region competent to respond to those signals, as well as

having the appropriate assay to detect the response. This project started from the initial idea that it may be possible to identify organizers prospectively, based on their expression of a set of common genes. Synexpressed genes are a group of genes that are co-ordinately expressed at one site (Gawantka et al., 1998; Niehrs and Pollet, 1999; Peter and Davidson, 2009; Tomancak et al., 2002). If there is such a common set of genes that characterises known organizers, their pattern of expression could be used as a diagnostic for discovering new organising centres. Specifically, genes enriched in known organizers should be enriched in candidate regions, whereas genes that are not expressed in organizers but expressed on other similar (non-inducing/patterning) tissues should be absent from the candidate regions. This is not easy to assess by simple observation partly because of the sheer number of genes involved and the complexity of development, and especially because it is difficult to "see" regions that do not express a particular gene.

To progress in this direction we (in collaboration with Prof. David Burt, Dr. Megan Davey and Dr. Frances Wong from Roslin Institute, Prof. Richard Baldock, Dr. Duncan Davidson, Dr.Jeff Chrisitansen and Mike Wicks from HGU, Prof. Cheryll Tickle and Dr.Monique Welten from Bath university, Dr. Paula Murphy from Trinity College Dublin) decided to construct a detailed, 3-dimensional atlas of gene expression which would then be analysed by image comparison software to define regions with similar expression characteristics for many genes at the same time. To help identify regions that have conserved patterns of synexpression of these genes between chick and mouse, the consortium (see above) decided to host the chick atlas of gene expression on the same server as an atlas of gene expression for the mouse (EMAGE; http://www.emouseatlas.org/emage/) . The EMAGE project started several years ago to act as a comprehensive repository of gene expression patterns during development, along with a detailed anatomical atlas and an anatomical ontology (Baldock et al., 2003; Ringwald et al., 1994). These are fully searchable by gene name, structure name, or location/pattern (drawn interactively by the user), and importantly, allowing complex Boolean queries (for example: "find genes expressed in structure A that are also expressed in structure B but not expressed in structure C at stage D of development"). During the course of this

project, a prototype chick atlas (eChickAtlas; http://www.echickatlas.org/ecap/home.html) was generated, providing some of the above functionality (Wong et al., 2013). To start to populate the atlas with data, the consortium decided to prioritise genes expressed during limb development (collected by the groups of Prof. Cheryll Tickle and David Burt) and the "synexpressed" set of genes identified from three of the known organizers: Hensen's node, the notochord and associated ventral neural tube and the ZPA in the posterior limb bud. This "synexpressed" set would include both genes that are enriched in and those that are depleted from these organizers, as compared to similar tissues at equivalent stages of development.

This following chapter describes the design of the screen and the identification of a set of genes expressed similarly in the three chosen organisers.

1.5.1 Microarray screen to identify a "synexpression" group of genes from three known organizers

(experments in this section were performed by Prof. David Burt group)

Figure 1.5 shows the tissues collected for microarray assays analysis. The gene expressions in organizers were compared to their closely-related, non-organizing regions. The following comparisons were performed. First, tissues were collected from HH3+ Hensen's node and the gene expression levels were compared to that in the posterior primitive streak at the same stage (Figure 1.5 A). The early (HH3+) node expressions were also compared to that in late node at HH6, which is not organizer anymore but retains some signalling activities like left-right patterning (Levin et al., 1995). The expressions in late node HH6 were also compared to that in early posterior primitive streak at HH3+ to further select genes expressed for signalling activities. Moreover, the expressions in the ventral neural tube and notochord were compared to that in the dorsal neural tube to select genes involved in notochord development (Figure 1.5 B, (Tanabe and Jessell, 1996). Finally, the expressions in posterior limb bud including ZPA were compared to that in the anterior limb bud both at early and late limb (HH20 and HH24 respectively), to

identify genes involved in limb development (Figure 1.5 C, (Zeller et al., 2009). The initial comparisons between organizer and their related non organizing regions select a large number of genes that were enriched or depleted in each organizer as it is shown in Table 1.1. To further narrow down the genes of interest and select synexpression groups, cross comparisons between each group in Table 1.1 were performed. Detail comparisons are shown in Table 1.2. At the end, genes were selected from comparison group 15, 16, 17 and 18, which represents genes that are enriched or depleted in early node and/or late node and the ventral neural tube and notochord and ZPA.

The final set of synexpressed genes selected is shown in Table 1.3. It includes transcription factors and intracellular molecules, which are expressed or depressed in cells within known organizers. It also includes membrane and secreted molecules, which are responsible for signalling activities to surrounding tissues. In total, 31 genes selected are enriched in organizers tested and 16 are depleted instead. In summary, we selected a set of genes, which are up- or down-regulated in current known organizers (Hensen's node, notochord and floor plate, ZPA), by comparing the gene expression level between organizers and their closely-related, non-organizer regions. These genes, therefore, may be characteristic of organizers and the region where they are commonly up- or down-regulated may be a potential organizer. However, microarray assays do not give spatial and temporal gene expression patterns and the expression levels of these synexpressed genes are needed to be further analyzed by other methods such as whole-mount *in-situ* hybridisation.

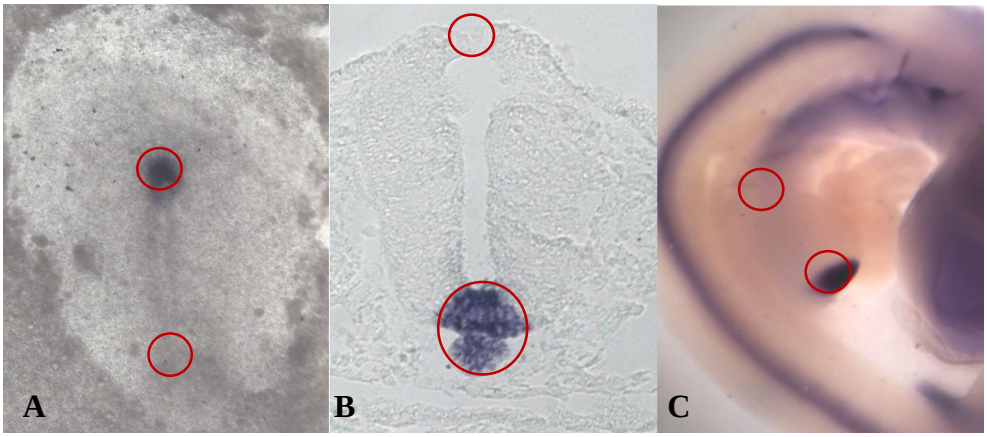


Figure 1.5 Tissues collected to perform microarray assays. The expressions of SHH are shown in all three pictures. A) Tissues were collected from HH3+ Hensen's node and posterior primitive streak at the same stage. Tissues were also collected from HH6 Hensen's node which is not shown here. B) Tissues were collected from the ventral neural tube and notochord together. Tissues from the dorsal neural tube at the same stage were collected as well. C) Tissues were collected from the posterior limb bud including the ZPA and anterior limb bud. Microarray assays were performed by Prof. David Burt's group at Roslin Institute to test the gene expression levels in each tissue.

Group	Comparison	Statistical test	UP_ probes	DOWN_ probes
1	S3HN_S3PS	FC >1.5; FDR <=0.05	1678	1415
2	S6HN_S3PS	FC >1.5; FDR <=0.05	3285	2075
3	S3HN_S6HN	FC >1.5; FDR <=0.05	1701	2819
4	VNT_DNT	FC >1.5; FDR <=0.05	811	283
5	S20LP_S20LA	FC >1.5; FDR <=0.05	227	361
6	S24LP_S24LA	FC >1.5; FDR <=0.05	760	1823

Table 1.1 The table shows the comparison of gene expression levels in organizers and their closely related, non-organizing regions. The column of "comparison" shows the two pieces of tissues collected to compare their gene expression levels by microarray assay. S3HN: early node collected between HH3+ and HH4. S3PS: posterior primitive streak collected between HH3+ and HH4. S6HN: late node collected between HH5 and HH6. VNT: ventral neural tube and notochord. DNT: dorsal neural tube. S20LP: posterior limb bud (ZPA) collected from HH20. S20LA: anterior limb bud collected from HH20. S24LP: posterior limb bud (ZPA) collected from HH24. S24LA: anterior limb bud collected from HH24. All genes are selected to be enriched or depleted in each group under the criteria of fold change (FC) bigger than 1.5 and false discovery rate (FDR) equal or smaller than 0.05. UP_probes column shows the number of genes enriched in the organizers or signalling centres. On the other hand, DOWN_probes column shows the number of genes depleted in the organizers or signalling centres.

Group	Comparison	Statistical test	UP_probes	DOWN_probes
1	S3HN_S3PS	FC >1.5; FDR <=0.05	1678	1415
2	S6HN_S3PS	FC >1.5; FDR <=0.05	3285	2075
3	S3HN_S6HN	FC >1.5; FDR <=0.05	1701	2819
4	VNT_DNT	FC >1.5; FDR <=0.05	811	283
5	L20P_L20A	FC >1.5; FDR <=0.05	227	361
6	L24P_L24A	FC >1.5; FDR <=0.05	760	1823
7	1 AND 3	FC >1.5; FDR <=0.05	614	532
8	1 AND 2	FC >1.5; FDR <=0.05	872	639
9	7 AND 4	FC >1.5; FDR <=0.05	66	17
10	7 AND 5	FC >1.5; FDR <=0.05	21	16
11	7 AND 6	FC >1.5; FDR <=0.05	28	36
12	8 AND 4	FC >1.5; FDR <=0.05	127	61
13	8 AND 5	FC >1.5; FDR <=0.05	25	21
14	8 AND 6	FC >1.5; FDR <=0.05	54	51
15	7 AND 4 AND 5	FC >1.2; FDR <=0.05	6	1
16	7 AND 4 AND 6	FC >1.2; FDR <=0.05	13	5
17	8 AND 4 AND 5	FC >1.2; FDR <=0.05	10	3
18	8 AND 4 AND 6	FC >1.2; FDR <=0.05	19	15
19	4 AND 5	FC >1.2; FDR <=0.05	48	29
20	4 AND 6	FC >1.2; FDR <=0.05	105	53
21	10 OR 11	FC >1.2; FDR <=0.05	78	88
22	13 OR 14	FC >1.2; FDR <=0.05	147	102
23	15 OR 16	FC >1.2; FDR <=0.05	14	7
24	17 OR 18	FC >1.2; FDR <=0.05	22	15
25	19 OR 20	FC >1.2; FDR <=0.05	124	61

Table 1.2 Cross comparisons of gene expressions between organizers. The first 6 groups were the original data set described in Table.3.1. The number in "Comparison" column from group 7 to 25 shows the comparison between which groups. For example, group 7 shows the data of comparison between group 1 and group 3. Genes selected at the end were under the criteria of fold change (FC) bigger than 1.2 and faults discovery rate equal or smaller than 0.05 (Groups 15, 16, 17 and 18). UP_probes and Down_probes columns show the genes enriched or depleted in organizer or signaling centers respectively after each comparison.

Up-regulated genes (31)	Transcription Factors	cNOT1, LMO7
	Intracellular molecules	GNPDA1, KCNMA1, KIRREL3, MCF2L, PKIγ, PLK1S1, PIK3CD, PIK3R5, PPP1R14C, SLC39A11
	Membrane or secreted molecules	ADRA2A, CHGA, ENPP4, FLBN7 LIKE, NXPH1, NRP1, NRSN1, PCSK6, PRKG1, PROM1, RAB11FIP4, SMOC1 (ChEST21m16), SHH, TMEM63C, TMTC2, TSPAN6, VTN
	unannotated	ChEST125i5, ChEST851k1
Down-regulated genes (16)	Transcription Factors	DLX5, DLX6, ID2, LDB2, MSX1, MSX2
	Intracellular molecules	BTG2, DSP, MOXD1, SOCS2
	Membrane or secreted molecules	CBLN2, CXCL12, EFNA5, PLXNC1, TNFRSF19
	unannotated	ChEST378m14

Table 1.3 this table shows synexpressed genes selected based on the comparisons of gene expression level between organizers and their close-related, non-organizing regions. Up-regulated genes are those enriched in organizers and down-regulated genes are those depleted in organizers. In total, 31 up-regulated genes and 16 down-regulated genes were selected. Both of them include transcription factors and intracellular molecules that are express during organizing activities and function in the cells within organizer regions. On the other hand, membrane and secreted molecules were also selected, which interact with or diffuse to surrounding non-organizer tissues. Some genes are un-annotated and are not studied before.

1.5.2 Distilled list genes

As described above, we have selected 47 genes that may be involved in organizing activities based on our microarray assays. Some of these genes like *SHH* are well known organizer genes. Some of them are known to be involved in many signalling activities such as CXCL12. But some are not studied in the developmental process and some even do not have an official gene name. The following chapter will generally introduce current research work for each gene involving known expression patterns and functions in the order as in Table 1.3.

- cNOT1

cNOT1 (Stein and Kessel, 1995), which is also called *gNOT1*(Ranson et al., 1995), belongs to a noto homeobox gene family. Its homolog is also found in xenopus (*Xnot1* and *Xnot2*) (Gont et al., 1993; von Dassow et al., 1993), zebrafish (floating head *flh*) (Talbot et al., 1995) and mouse (Abdelkhalek et al., 2004; Plouhinec et al., 2004). There is also another gene called *cNOT2* that is located right next to *cNOT1* and has a similar expression pattern in early embryo that has been reported before (Stein et al., 1996). Based on mutations and gain-of-function experiments, homologs of *NOT* gene in other animals have been shown to be involved in notochord development (Abdelkhalek et al., 2004; Gont et al., 1993; Halpern et al., 1995; Talbot et al., 1995). Also, in zebrafish, it is shown to be involved in the development of the pineal gland as its mutation affects the maturation of pineal cells (Snelson et al., 2008).

- LMO7

Lim domain only protein 7 (Lmo7) is a transcription factor and is a LIM/PDZ domain containing protein (Semenova et al., 2003). It was previously reported to be involved in myogenic differentiation through regulation of Emerin in mouse (Dedeic et al., 2011; Lindvall et al., 2005). Down-regulation of *LMO7* in HeLa

Cells reduced emerin mRNA expression as well as other muscle-relevant genes, suggesting Lmo7 is required for the regulation of transcription of those genes (Holaska et al., 2006). Also, knockdown of *LMO7* with morpholinos in zebrafish caused a heart defect (Ott et al., 2008).

- GNPDA1

Glucosamine-6-phosphate deaminase 1 (GNPDA1) was first identified as a soluble hamster sperm protein that correlated with the calcium oscillations for the activation of egg and embryo development and it was later found in human as well (Parrington et al., 1996; Shevchenko et al., 1998). In mouse, *GNPDA1* mRNA was detected widely, including testis, heart, liver, kidney and lung (Amireault and Dube, 2000). The expression of *GNPDA1* has not been studied in chick embryo yet.

- KCNMA1

Potassium large conductance calcium-activated channel, subfamily M, alpha member 1 (KCNMA1) is also known as slo and slo1 (Cui et al., 2009b; Magleby, 2003; Salkoff et al., 2006). Its activation depends on the concentration of intracellular Ca^{2+} and mediates export of potassium (Brenner et al., 2000; Meera et al., 1996). Therefore it is expressed widely and has diverse physiological functions including regulations of blood flow, immunity and neurotransmission (Horii et al., 1994; Lu et al., 2006; Morton et al., 2004). In chick embryo, it is found to be expressed in retina and cochlea (Rosenblatt et al., 1997).

- KIRREL3

Kin of IRRE like 3 is also known as Neph2 and is involved in a transmembrane family of Kirrel that contains immunoglobulin domain (Volker et al., 2012). It is widely expressed in the nervous system in mouse and chick and is shown to be

responsible for the CNS patterning (Gerke et al., 2006; Volker et al., 2012). For instance, *KIRREL3* mutant mice shows an abnormal structure of accessory olfactory bulb (Prince et al., 2013).

- MCF2L

MCF.2 cell line derived transforming sequence-like (MCF2L) is also known as DBS or OST (Horii et al., 1994; Yamauchi et al., 2002). It is found as an oncogene that interacts with Rac1, RhoA and Cdc42 and is expressed high in brain and neurons in rats (Horii et al., 1994). Although its role in development is not clear yet.

- PKIγ

Protein kinase (cAMP-dependent, catalytic) inhibitor gamma belongs to the family of inhibitors of cAMP-dependent protein kinases (PKAs) (Collins and Uhler, 1997). The other two members in this family are *PKI*$_\alpha$ and *PKI*$_\beta$ (Doskeland et al., 1993; Francis and Corbin, 1994) . The expression patterns of these isoforms are significantly different. *PKI*$_\alpha$ is found to be expressed in heart, skeletal muscle, cerebral cortex and cerebellum in mouse (Olsen and Uhler, 1991; Van Patten et al., 1997). It is also found to be expressed asymmetrically in the node of chick embryo, which suggests its role in left-right asymmetry (Kawakami and Nakanishi, 2001). Interestingly, *PKI*$_\beta$ mRNA is detected in mouse testis (Van Patten et al., 1997). *PKI*$_\gamma$ is also found to be predominantly expressed in mouse heart (Collins and Uhler, 1997). Expressions studies in quail shows that these isoforms of *PKI* are overlap expressed in heart and skeletal muscle (Wakamatsu, 2009).

- PLK1S1

Polo-like kinase 1 substrate 1 is a homolog to Gm411 in mouse and Kizuna in human (Oshimori et al., 2006; Tang et al., 2008). Polo-like kinases (PLKs) are conserved proteins that are responsible for the activation of cyclin-dependent kinase

1-cyclin B and thus for regulation of cell cycle progression (Nigg, 1998; van de Weerdt and Medema, 2006). *Plk1*$^{-/-}$ mice is embryonic lethal due to failure to progress through the mitotic phase, confirming the role of Plk1 in cell cycle regulation (Lu et al., 2008a). Plk1s1 is a centrosomal substrate of Plk1 and depletion of Plk1s1 by siRNA results multipolar spindles in transfected HeLa cells (Oshimori et al., 2006). Also, Plk1s1 is homologues to Drosophila bam, the activation of which causes germ cells differentiation. Similarly, *Plk1s1* is highly expressed in differentiating germ cells and in differentiated cells of adult testis in mouse (Tang et al., 2008).

- PIK3CD

Phosphoinositide-3-kinase, catalytic, delta polypeptide (PIK3CD) is also known as p110δ (Kok et al., 2009). It is a member of a lipid kinase family that is involved in signal transduction through tyrosine kinase- and heterotrimeric G-protein-coupled receptors (Herman and Johnson, 2012). *PIK3CD* is regulated to express in leukocytes, while p110α and β are widely expressed (Bi et al., 2002; Geering et al., 2007; Vanhaesebroeck et al., 1997). P110δ is associated with the p85α and β adaptor subunits as p110α (Vanhaesebroeck et al., 1997). In *p110δ*$^{-/-}$ mice, there is a reduction of peripheral B cells and a B1 B-cell deficiency (Jou et al., 2002).

- PIK3R5

Phosphoinositide-3-kinase, regulatory subunit 5 (PIK3R5) is also known as p101. It is involved in the phosphorylation of membrane lipids that leads to the activation of the AKT pathway (Al Tassan et al., 2012). The role of PIK3R5 in early embryo development is not clear yet and its expression pattern has not been studied in any animal yet.

- PPP1R14C

Protein phosphatase 1, regulatory (inhibitor) subunit 14C (PPP1R14C) is also known as KEPI. It is an inhibitor for type 1 Ser/Thr protein phosphatises and regulates ERK signalling pathway (Wenzel et al., 2007). PPP1R14A, which is other subunit of the same regulatory pathway, is expressed in the gut endoderm at E8.5 mouse embryo (Tamplin et al., 2008). However the detail of the role of PPP1R14C in the development of the embryo is not still clear.

- SLC39A11

Solute carrier family 39 member 11 is also known as ZIP11, which is involved in a large ZIP family of metal ion transporters (Kambe et al., 2004). It is located at the plasma membrane and differs from other members in ZIP family as it lacks the zinc-binding site His-rich loop (Kambe et al., 2006). Its role in the development of the embryo is not clear.

- ADRA2A

Adrenergic alpha-2A-receptor (ADRA2A) is a conserved G protein-coupled receptor in the CNS found in chick and rat brain (Diez-Alarcia et al., 2009; Fernandez-Lopez et al., 1990). The mRNA of *ADRA2A* is detected in the interdigital mesenchyme during digit separation in mouse and transfection of ADRA2A into mesenchymal cell lines displaces accelerated apoptosis (Wang and Limbird, 1997). *ADRA2A* is also expressed widely in craniofacial regions and in the central nervous system both in mouse and chick embryos (Diez-Alarcia et al., 2009; Wang and Limbird, 1997) Double knockout of *ADRA2A/2C* in mouse embryos causes alteration of distribution of cortical interneurons suggesting its role in the development of cortical circuits (Riccio et al., 2012).

- CHGA

Chromogranin A (parathyroid secretary protein 1) are polypeptides found in secretory granules in neurons and endocrine cells (Iacangelo and Eiden, 1995). It is a precursor of several active smaller peptides including pancreastatin, vasostatin-I, catestatin and parastatin (Aardal and Helle, 1992; Fasciotto et al., 1993; Koeslag et al., 1999; Tatemoto et al., 1986). It was detected by immunochemistry in chicken pars tuberalis of the pituitary gland early at 6 days of incubation and in the intestinal tract from day 10 (Kameda et al., 1998; Salvi et al., 1996). In zebrafish, it starts to be expressed 16 hours post fertilization and is highly expressed.

- ENPP4

Ectonucleotide pyrophosphatase/phosphodiesterase (ENPP) is a conserved ectonucleotidase that can catalytically interact with nucleotides and their derivatives (Zimmermann, 1999). ENPP4 is detected in many tissues as the other ENPP members (Bollen et al. 2000), but little is known about its catalytic function. In *Xenopus*, the expression of *ENPP4* is detected in blastomeres of the animal pole, cement gland, pronephric tubles, somites and kidney. Its expression is aslo upregulated during neurulation (Masse et al., 2010).

- FBLN7L

Fibulin 7 like (FBLN7L) is homologous to fibulin 7 (FBLN) in mouse and human (data in our lab). It is also known as TM14 (de Vega et al., 2007). It was first identified as an extracellular protein expressed in teeth, cartilage, hair follicles and extraembryonic tissues of the placenta in mouse embryos (de Vega et al., 2007). It is shown to interact with molecules including heparin, fibronectin, fibulin-1 and dentin sialophosphoprotein in the extracelluar matrix and it also binds to dental mesecenchyme cells and odontoblasts (de Vega et al., 2007).

- NXPH1

Neurexophilin 1 (NXPH1) belongs to the neurexophilin family and is a secreted glycoprotein that binds to α-neurexins (Missler et al., 1998). The neurexophilin gene was expressed highly in adult rat and mouse brain testing by *in-situ* hybridisation (Petrenko et al., 1996). In chick, it was detected in paravertebral sympathetic ganglia by RNA *in-situ* hybridisation at E18 embryos (Apostolova et al., 2007).

- NRP1

Neuropilin 1 (NRP1), which is also known as membrane protein A5, is a vertebrate specific transmembrane protein found in blood vessels and nerves (Hirata et al., 1993; Takagi et al., 1995). It was first identified in the visual and somatic sensory neurons in *Xenopus* and was shown to promote neurite outgrowth by culture of retinal explants or ganglion neurons with *A5*-expressing cells (Hirata et al., 1993). On the other hand, the chick homologue of neuropilin is restricted to express in cells receiving retinal input in the optic tectum and amacrine cells of the retina as well as growing neurites of cultured dorsal root ganglia, suggesting that chick neuropilin is involved in the development of certain neuronal circuits *in vivo* (Takagi et al., 1995). Furthermore, cell aggregation and functional knock down assays suggest that chick neuropilin functions as a adhesion molecule for neural crest cell migration (McLennan and Kulesa, 2007). In addition, neuropilin-1 is shown to be involved in blood vessel development. Amino acid sequence shows that it is a receptor for vascular endothelial growth factor VEGF-A (also called VEGF165 or VEGF164I in mice) and increases the affinity of VEGF-A for VEGFR2 for cell migration (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997; Migdal et al., 1998; Soker et al., 1998). Alternatively, SEMA3A competing with VEGF-A binds to NRP1 for vascular growth (Appleton et al., 2007; Miao et al., 1999). Therefore, overexpression of *NRP1* in mice leads to overgrown of capillaries and blood vessels (Kitsukawa et al., 1995), while targeted inactivation of NRP-1 leads to vascular defects (Kawasaki et al., 1999).

- NRSN1

Neurensin 1 is a membrane protein that is expressed in neurons and in the developing retina of mouse, especially in the growth cones of actively extending neurites (Kadota et al., 1997; Nagata et al., 2006). The neurensin protein is abundant in the growing distal end of neurites and transfection of neurensin causes epithelial cells to become neuron-like, which suggests neurensin may play a role in neurite extension during development (Araki et al., 2002; Ida et al., 2004). Also it is up-regulated in the early regeneration of motor nerves after crush injury in mouse and thus suggests its roles in regeneration (Suzuki et al., 2007).

- PCSK6

Proprotein convertase subtilisin/kexin type 6 (PCSK6) is also known as PACE4 or SPC4, which is a proprotein convertase for proteolytic maturation of many proteins (Creemers et al., 1993; Mains et al., 1997; Seidah et al., 2008). Constam and Robertson shows that SPC4-dificient mouse develops situs ambiguus and altered expression patterns of *Nodal*, *Lefty* and/or *Pitx2*, which demonstrates that SPC4 is involved in left-right axis formation. They also demonstrates that SPC4 is involved in anterior-posterior axis formation using chimeric embryos and immunochemistry (Constam and Robertson, 2000).

- PRKG1

Protein kinase, cGMP-dependent, type I (PRKG1) is an intracellular receptors for second-messenger cGMP (Hofmann et al., 2009). It contains two isoform, type Iα and type Iβ recognized in human tissues (Orstavik et al., 1997). The antibody against both human PRKG Iα and Iβ labelled the smooth muscle layer of the aorta and pericyctes encircling capillaries in the lateral wall of guinea pig cochlea (Tian et al., 1999). In mouse embryo, *PRKG1* expression is observed in dorsal root ganglion neurons, the spinal floor and roof plates (Qian et al., 1996). *PRKG1* expressed with *Pde5* in cochlear hair cells and deletion of *PRKG1* in mice results in

less recovery from noise-induced hearing loss, suggesting *PRKG1* is involved in hearing function (Jaumann et al., 2012). In addition, induction of cGMP analogs to chick neural plate explants can promote its response to Shh (Robertson et al., 2001), while inhibition of cGMP by inhibitor DT-2 reduces the Shh response in mouse embryonic stem cells (Christensen et al., 2006), suggesting there is a link between Shh signalling and PRKG1.

- PROM1

Prominin 1 (Prom 1, also known as CD133) is a cholesterol-interacting transmembrane glycoproteins that locates to the apical microvilli and primary cilia of neuroepithelial and glandular cells and is involved in neuroepithelial cell differentiation (Corbeil et al., 2010; Weigmann et al., 1997). Loss of *Prom1* in mice causes a deficiency in retinal development and blindness (Zacchigna et al., 2009).

- RAB11-FIP4

RAB11 family interacting protein 4 (RAB11-FIP4) is a member of the Rab11-FIP family that interacts with Rab11 in a GTP-dependent manner (Zerial and McBride, 2001). In zebrafish embryos, it is expressed in retina progenitors and later in the ganglion cell layer and ciliary marginal zone, while Rab11-FIP4A knockdown embryos and rescue experiments by ectopic expression of either *p57Kip2* or dominant-negative PKA show that *RAB11-FIP4A* is involved in retinal development by regulating Shh signalling (Muto et al., 2006). Similarly, mouse *Rab11-FIP4* is also expressed in neural tissues and gain- and loss-of function experiments show that it plays a role in cell cycle control of retinal cells with Shh signalling (Muto et al., 2007).

- SMOC1

Secreted modular calcium binding 1 (SMOC1) is a secreted glycoprotein belonging to the matricellular protein SPARC family, which is a group of extracellular

proteins that regulates various cellular functions without contributing to physical structures (Bornstein, 1995; Bornstein and Sage, 2002; Vannahme et al., 2002). *SMOC1* is expressed widely in organ basement membranes and in node, mesoderm, somite, otic vesicle and limbs in embryonic stages of mouse (Okada et al., 2011; Tamplin et al., 2008; Vannahme et al., 2002) Gain of function of *SMOC1* in *Xenopus* shows a consistent result as a BMP antagonist, while complete loss of function of *SMOC1* results in arrest of development before neurulation (Thomas et al., 2009). SMOC1 is acting downstream of BMP signalling, one evidence is that SMOC1 cannot induce neural markers if inhibition of BMP signalling through phosphorylation of Smad is prohibited (Thomas et al., 2009). In addition, in *SMOC1* null mice, expression of genes within BMP signalling is disturbed and phenotypes of microphthalamia with limb anomalies (MLA) are present, suggesting SMOC1 is involved in ocular and limb development (Okada et al., 2011). The equivalent mutation in human *SMOC1* causes ophthalmo-acromelic syndrome (OAS) which is a combination of eye and limb malformations (Rainger et al., 2011).

- SHH

Shh is from the vertebrate hedgehog family, which includes other two members Dersert hedgehog (Dhh) and Indian hedgehog (Ihh). The hedgehog gene is first identified in Drosophila and was later found in different animals including sea urchin, mouse, zebrafish, rat, chicken and human (Varjosalo and Taipale, 2008). Shh binds to the transmembrane protein receptor Patched (Goodrich et al., 1996; Marigo et al., 1996) and leads to the activation of target genes including transcription factor HNF3β (Sasaki et al., 1997) through zinc finger transcription factors Gli (Alexandre et al., 1996; Ding et al., 1998; Ruiz i Altaba, 1997).It can act both as contact-dependent or as a diffusible morphogen (Chuang and McMahon, 1999; Johnson and Tabin, 1995). This gene was selected as a positive control in the microarray assay. *SHH* is expressed asymmetrically in the left side of the late node and is responsible for the left-right axis patterning (Cui et al., 2009a). It is also expressed in the notochord and floor plate to specify motoneurons in the ventral neural tube (Ericson et al., 1996; Marti et al., 1995; Placzek et al., 1991; Ruiz i

Altaba et al., 1995). *SHH* is also expressed in the ZPA for anterior and posterior patterning in limb bud (Riddle et al., 1993b).

- TMEM63C

Transmembrane protein 63C (TMEM63C) is a protein that has not been studied before.

- TMTC2

Transmembrane and tetratricopeptide repeat containing 2 (TMTC2) is a transmembrane protein with unknown function. It is downregulated by both HIF-1α and HIF-2α siRNA (Mole et al., 2009), suggesting it is regulated by transcription factor hypoxia-inducible factor HIF-1α and HIF-2α (Pouyssegur et al., 2006; Wenger, 2002; Wiesener et al., 1998). And it is upregulated when neurogenin-1is overexpressed in human neural stem cells (Satoh et al., 2010).

- TSPAN6

Tetraspanin 6 is also called TM4SF6, belonging to the tetramembrane 4 superfamily (TM4) (Hemler, 2005; Maeda et al., 1998). Other members of tetraspnin family have been shown to interact with various types of proteins including integrins and membrane receptors and are involved in different cellular process like cellular signalling, adhesion and fusion (Hemler, 2005; Maecker et al., 1997; Yunta and Lazo, 2003). *TSPAN6* mRNA is expressed widely in adult human tissues (Maeda et al., 1998; Todd et al., 1998). Although functions of TSPAN6 in early embryo development are not clear, studies have been done on other members of the TSPAN family. *TSPAN1* in *Xenopus* is found to be expressed in the dorsal ectoderm and neural plate and knockdown experiment revealed that it is involved in gastrulation movements and primary neurogenesis (Yamamoto et al., 2007). *TSPAN5* mRNA is highly expressed in the cortical areas and Purkinje cells in mouse embryo during development (Garcia-Frigola et al., 2001). Finally, some

members of tetraspanin are highly expressed in the embryonic chick spinal cord including NAG-2 (homologous to human TSPAN4), cd9 (homologous to human TSPAN29) and TSPAN18 (Perron and Bixby, 1999).

- VTN

Vitronectin (VTN) a glycoprotein that presents in the extracellular matrix both in adult and embryonic tissues (Preissner, 1991). It is induced by Shh during the differentiation of motor neurons in the notochord and floor plate and also the development of retina in chick (Martinez-Morales et al., 1995; Pons and Marti, 2000). Its involvement in CNS development is also observed in the mouse embryo (Seiffert et al., 1995).

- ChEST125i5

ChEST125i5 is unannotated gene that has not been studied yet.

- ChEST851k1

ChEST851k1 is another unannotated gene that has not been studied yet.

- DLX5 and DLX6

DLX homeobox genes are homologous to the Drosophila Distal-less (*Dll*) gene, and are transcriptional activators (Panganiban and Rubenstein, 2002). There are 6 *DLX* genes in mammals and eight in the zebrafish (Ekker et al., 1992; Ellies et al., 1997; Panganiban and Rubenstein, 2002; Stock et al., 1996). *DLX5* and *DLX6* are paired to convergently transcribe, hence their expression patterns are similar to each other (Stock et al., 1996; Zerucha et al., 2000). Robledo and his colleagues show that *DLX5/6*$^{-/-}$ mice have severe craniofacial, axial and appendicular skeletal

abnormalities and overexpression of *DLX5* can rescue limb development in *DLX5/6* null mice (Robledo et al., 2002). In addition, the *Msx1/2:DLX5:DLX6* triple knock-out mice show defects in hindlimb, while single mutation of any of them dos not lead to limb defects, suggesting interactions between Msx and Dlx molecules (Vieux-Rochas et al., 2013). Also, Bmp7, Bmp4 and Bmp antagonist Follistatin are misexpressed in the palate of *DLX5*$^{-/-}$ embryos, suggesting Dlx5 is involved in regulation of BMP signallings (Levi et al., 2006). Finally, in chick embryo, ectopic expression of *DLX5* inhibits lens-specific gene expression (Bhattacharyya et al., 2004), while otic progenitors expresses *DLX5* based on fate mapping and gene expression analysis (Streit, 2002).

- ID2

Inhibitor of DNA binding 2 (ID2) is a dominant negative helix-loop-helix (HLH) transcriptional regulator that is involved in many developmental processes (Martinsen and Bronner-Fraser, 1998). There are four members in ID family (ID1 to ID4). Apart from ID1, they all have a conserved sequence SPVR for phosphorylation of cyclin E-cdk2 and cyclin A-cdik2 kinases. Hence Id proteins play roles in the regulations of proliferation, differentiation and apoptosis (Butler et al., 2009).Brad J. Marinsen and Marianne Bronner-Fraser showed that *ID2* is expressed in cranial and migrating cranial neural crest cells in chick embryo. Ectopic expression of *ID2* converted ectodermal cells to neural crest fate and overexpression of *ID2* resulted in overgrowth and premature neurogenesis of the dorsal neural tube. Hence ID2 is involved in regulation of neural crest cells to become neural rather than epidermal lineages (Martinsen and Bronner-Fraser, 1998). Genes of Id are also targets of BMP signals, for example, inhibition of BMP receptors blocks the expression of *ID2* in mouse myoblast cells and CGCC sequence is identified as a Smad target by a reporter assay (Nakahiro et al., 2010).

- LDB2

LIM domain binding 2 (Ldb2) is also known as Clim1. It is a nuclear cofactor for LIM homeodomain transcription factors (LIM-HDs) and LIM-only proteins

(LMOs), which can potentiate gene activation or inactivation (Bach, 2000; Sanchez-Garcia and Rabbitts, 1994; Tran et al., 2006) This LIM-domain binding protein family includes another member which is Ldb1 (Clim2). Expressions of *LDB1* and *LDB2* mRNA are similar in mouse embryo and are detected in telencephalon, mesencephalon, metencephalon, neural tube, dorsal root ganglia and limbs (Bach et al., 1997). Targeted deletion of the *LDB1* gene in mouse embryo causes defects in heart, head and extraembryonic strucutures, which may be due to misexpression of Wnt antagonists (Mukhopadhyay et al., 2003) Also, Ldb1 interacts with Lhx3 and is involved in motor neuron and interneuron differentiation (Thaler et al., 2002) as well as development and function of the pituitary gland (Susa et al., 2006).

- Msx1 and Msx 2

Msh homeobox gene is a transcriptional repressor that was initially identified as homologous to the Drosophila muscle segment homeobox gene (*msh*) (Hill et al., 1989; Robert et al., 1989). This family contains 3 unlinked members including Msx1, Msx2 and Msx3. Apart from *MSX3* that is expressed in the dorsal neural tube only (Shimeld et al., 1996), *MSX1* (also called Hox7) and *MSX2* (also called Hox8) are widely expressed in embryonic development. Their expressions are overlapping with each other, especially in the developing craniofacial regions (Alappat et al., 2003). Also, *MSX2* is expressed in the limb bud mesoderm at early stages in a way that consistent with specification of anterior-posterior axis and is also expressed in the interdigital mesenchyme in later stages suggesting its involvement in apoptosis (Coelho et al., 1991). Conditional mutation of *MSX1* and *MSX2* in the limb bud mesoderm of mouse embryo shows anterior expression of *SHH* and misexpression of BMP molecules, suggesting it is required for proper Shh and BMP signalling in developing limbs (Bensoussan-Trigano et al., 2011). Further studies prove that *MSX* genes are downstream effectors of BMP signalling, for example, inhibition of BMP antagonist *Noggin* results in a reduction of *MSX2* expression (Brugger et al., 2004; Khokha et al., 2003; Marazzi et al., 1997). BMP

signalling is generally down-regulated in known organizers as discussed in Chapter 1.

- BTG2

B-cell translocation gene 2 (BTG2) is also known as Tis21, Pc3 or APRO1. *BTG2* is an anti-proliferative gene involved in regulation of the cell cycle and response to DNA damage. It is homolog to rodent *Pc3/Tis21*, which is induced by p53. BTG2 shares similar sequence with BTG1, which is another member in the same family (Rouault et al., 1996). BTG2 is shown to be a coactivitor-corepressor and/or and adaptor molecule that interacts with protein arginine methyltransferase 1, Hoxb9 and Caf1 (Lin et al., 1996; Prevot et al., 2001; Prevot et al., 2000). *BTG2* is expressed in mouse presomitic mesoderm, tail bud and somites and is involved in vertebral patterning as *BTG2* null mice display posterior homeotic transformations caused by impaired BMP/Smad signalling (Park et al., 2004). *BTG2* is also expressed in the neural plate border, presomitic mesoderm, trigeminal placode and mesonephros in early chick embryo (Kamaid and Giraldez, 2008).

- DSP

Desmoplakin (DSP) is a cytolinker protein and is a key component of desmosomes, which are intercellular junctions of epithelial tissues and cardiac muscles (Al-Jassar et al., 2011; Kowalczyk et al., 1999). *DSP*$^{-/-}$ mice embryo cannot survive over E6.5 with major defects in heart muscle, neuroepithelium and skin epithelium due to loss or instability of desmosomes (Gallicano et al., 1998). Rescuing DSP function by supplementing with wild-type extra-embryonic tissues enables *DSP*$^{-/-}$ embryos to express desmosome-like structures and survive longer, showing the importance of DSP for normal function of demosome (Gallicano et al., 2001; Gallicano et al., 1998). Plakoglobin, which is another desmosome protein and negative regulator of canonical Wng signalling, is localized to the nucleus in DSP-deficient cells (by siRNA), while normally it is localized in the cytoplasm, leading to suppression of Wnt signalling and promotion of adipogenic and fibrogenic genes (Garcia-Gras et al., 2006) . The cardiac-restricted deletion of DSP shows heart dysfunction and

embryonic lethality that is consistent with previous studies by Gallicano (Gallicano et al., 1998; Garcia-Gras et al., 2006). Also, loss of function of DSP in combination with fluorescently labelled microtubules shows DSP is required for relocalization of the microtubule-anchoring protein ninein to desmosomes (Lechler and Fuchs, 2007). Furthermore, whole-mount *in-situ* hybridisation in mouse demonstrates that DSP is expressed in the visceral endoderm and the endoderm near the node at E7.5, and later on in the otic vesicle, eye, midgut and hindgut, pharyngeal region, somites and heart (Moore-Scott et al., 2007).

- MOXD1

Monooxygenase, DBH-like 1 (MOXD1) is also known as DBHR. Dopamin β-hydroxylase (DBH, also known as DBM dopamine β-monooxygenase) is included in copper monooxygenase family (Ikeno et al., 1977; Stewart and Klinman, 1988). DBHR (DBH-related) is 30% genetically similar to DBH (Knecht and Bronner-Fraser, 2001). DBH catalyzes the conversion of dopamine into neurotransmitter and norepinephrine and it is detectable from HH18 at chick embryo during the development of sympathetic neurons (Ernsberger et al., 2000; Stewart and Klinman, 1988). In comparison, *DBHR* is expressed in prospective neural crest cells at HH8. It is also expressed in the myotome, dorsal neural tube, rhombomeres and limbs (Knecht and Bronner-Fraser, 2001).

- SOCS2

Suppressor of cytokine signalling family (SOCS) regulates cytokine signal induction by inhibiting both phosphorylation of interleukin-6-induced receptor and activation of JAK/STAT pathways (Hilton et al., 1998; Starr et al., 1997). SOCS2 belonging to the SOCS family interacts with insulin-like growth factor-I receptor (IGF-IR) both in vitro and in vivo and it is also widely expressed in human fetal and adult tissues (Dey et al., 1998). *SOCS2*$^{-/-}$ mice have enlargement of most organs which due to deregulation of growth hormone and IGF-I (Dey et al., 1998; Metcalf

et al., 2000). *SOCS2* is also highly expressed in the developing nervous system from embryonic day 14 in mice (Polizzotto et al., 2000). *SOCS2*$^{-/-}$ mice has less neurons, while transfection of SOCS2 in *SOCS2*$^{-/-}$ mice or overexpression *SOCS2* in transgenic mice both promotes neurogenesis, suggesting that SOCS2 promotes neuronal differentiation (Scott et al., 2006; Turnley et al., 2002).

- CBLN2

Cerebellin 2 precursor (Cbln2) is a secreted glycoprotein belonging to the cerebellin family and is detected in adult and developing brain (Kavety et al., 1994; Miura et al., 2006; Wada and Ohtani, 1991). In the mouse brain, *CBLN2* mRNA is expressed in the olfactory bulb, thalamus, inferior colliculus, trigeminal spinal tract nucleus, neocortex and cerebral cortex (Miura et al., 2006). Cbln1, which is another protein in the cerebellin family, has a well-known function for bridging granule neurons to Purkinje cell in the cerebellum (Hirai et al., 2005; Matsuda et al., 2010). Expression of *CBLN2* in *CBLN1*-null mice can rescue the cerebellar defects, but *CBLN2*-null mice do not appear to have the same alteration in synaptic spine densities as in *CBLN1*-null mice, suggesting THAT Cbln2 has functional redundancy with Cbln1 in the cerebellum but not in the thalamus(Rong et al., 2012). In addition, *CBLN2* mRNA is detected in the distal forelimb and hindlimb in mouse embryo, and its expression is altered in *LMX1B* mutant embryo, which is a transcription factor for dorsoventral limb patterning (Gu and Kania, 2010). In chick embryo, *CBLN2* is expressed in synpatically connected neurons in the dorsal root ganglia and spinal cord (Reiner et al., 2011; Yang et al., 2010).

- CXCL12

Chemokine (C-X-C motif) ligand 12 (CXCL12) is also known as stromal cell-derived factor 1 (SDF1) (Read et al., 2005). Cxcl12 is a secreted ligand for the G protein-coupled Cxcr4 chemokine receptor and an orphan receptor, Cxcr7, and is involved in cell migration (Braun et al., 2002; Nishikawa et al., 1988; Nishimura et al., 1998; Shirozu et al., 1995; Tiveron and Cremer, 2008). During mouse

gastrulation, *CXCL12* is expressed in ectoderm and CXCR4 is expressed in mesoderm and endoderm (McGrath et al., 1999). Various types of defects are shown in *CXCL12* or *CXCR4* mutant mice including defects in the ventricular septum, cerebellum, retina, and gastrointestinal tract (Ma et al., 1998; Nagasawa et al., 1996; Tachibana et al., 1998; Zou et al., 1998). In chick embryo, *CXCL12* is expressed in ectoderm, sclerotome, intersomitic spaces and developing limbs (Rehimi et al., 2008).

- EFNA5

Ephrin A5 (EFNA5) encodes one of the nine ephrin ligands that bind to Eph receptor EPHA4. Both ephrin receptors and ligands are membrane proteins so they are involved in cell-cell contact and the signal is bidirectional (Pitulescu and Adams, 2010). Eph receptors and ligands are shown to be involved in cell positioning, migration, adhesion and differentiation in various tissues during development (Andersson et al., 2011; Andres and Ziemiecki, 2003; Feldheim and O'Leary, 2010; Pitulescu and Adams, 2010; Weiler et al., 2009). For example, EFNA5 enhances N-cadherin/β-catenin interactions and *EFNA5*$^{-/-}$ shows high percentage of cataracts due to disruption of lens fiber cell packing (Cooper et al., 2008). Gradient expression of *EFNA5* is also observed in organization of visual connections in chick embryo (Marin et al., 2001). Detailed analysis of *EFNA5* expression in early chick development shows that it is expressed from HH7 in the neural fold and in the head mesenchyme between HH9-12 (Baker and Antin, 2003). Also, EFNA5/EPHA4 ligand and receptor are both detected in lateral motor column of the HH21-28 spinal cord and dorsal root ganglia in chick embryo and the overlap of their mRNA expressions suggest a possible role in motor axon projections and pathfinding to the hindlimb (Eberhart et al., 2000).

- PLXNC1

Plexin C1 (PLXNC1) codes for a large single-pass transmembrane protein that interacts with semaphorin7A (Sema7A) (Tamagnone et al., 1999). The other members of the plexin family are shown to be involved in many different developmental processes, including development of the nervous system, regulation of axon guidance, cell motility and migration, and the immune response (Basile et al., 2005; Negishi et al., 2005; Renaud et al., 2008; Roney et al., 2013; Winberg et al., 1998). PLXNC1 is shown to be involved in neuronal migration and development in thed rat (Pasterkamp et al., 2007). In mice, *PLXNC1* is expressed in the optic recess of the diencephalon, meninges and olfactory lobe. It is also expressed in the lung mesenchyme, cargilarge primordium and endocardium and vascular endothelia. Later on, *PLXNC1* is expressed in the developing sex chords in the testis (Perala et al., 2005).

- TNFRSF19

TNFRSF19 is a member of the tumor necrosis factor receptor superfamily (TNFRSF) (Hu et al., 1999). It encodes a membrane receptor for the cytokine, lymphotoxin-α and the resultant signalling activates NFkB mediated transcription (Hashimoto et al., 2008). *TNFRSF19* mRNA is expressed mainly in skin epithelia and hair follicles in mouse (Hu et al., 1999; Kojima et al., 2000). But it is also expressed in the mouse embryonic limb bud, tooth, mammary glands and shiskers (Pispa et al., 2003). In chick embryo, it is expressed in feather placodes at E8 (Drew et al., 2007).

- ChEST378m14

ChEST378m14 is an annotated gene of unknown function.

1.6 Thesis plan

The above paragraphs briefly summarize what is known about five organizers that has both inducing and patterning activities on surrounding cells in early development: the "primary" organizer (Spemann organizer, Hensen's node), the notochord and floor plate, anterior neural ridge, isthmus and the ZPA. Considering the complexity of development, it seems very likely that other organizers must exist, to pattern the large diversity of embryonic territories that form. All known organizers have been discovered by transplantation experiments, which also require very good markers for the patterns affected by the organizer being tested in the neighbouring tissue, access to competent cells at the appropriate time in development and a certain amount of randomness in testing the correct signalling regions. Could there be another way, such as a strategy to predict which regions might act as organizers and might be worth testing by transplantation? That common properties (at least common signals) may characterise different organisers is suggested by the finding that transplantation of Hensen's node to the anterior aspect of the limb bud generates digit duplications as if it were a ZPA (Hornbruch and Wolpert, 1986)– of course this could be to Shh produced by notochord cells derived from the grafted node, but it also raises the possibility that other genetic components are conserved among organizers.

The underlying hypothesis behind the present project is that there may exist a group of genes common to all signalling regions, and which specify an "organizer state". By comparing the transcriptome of three of the known organizers (Hensen's node, notochord/floor plate and ZPA) with their closest non-organizer tissues at the same stage and with each other, we identified a set of about 50 genes that are either enriched or depleted in organizers (Chapter 1.5). This was used to explore the embryo for other sites sharing similar expression patterns, which could reveal novel organizers.

Therefore, the first goal of my project is to find out common sites of expressions of those distilled list genes, which may be potential organizers. The rest of the work in

the thesis provides some preliminary experiments required to test the putative organizing functions of one region sharing expression of some of the genes of the "synexpression group", the pineal region at the roof of the diencephalon.

Chapter 2
Method and material

2 Methods and material

2.1 Harvesting chick embryos

Gallus gallus chick was used in all experiments and eggs were incubated at 37˚C to required stages (Hamburger and Hamilton, 1992). Embryos were fixed in 4% paraformaldehyde in *poly (butylene succinate)* (PBS), pH 7.5. Embryos were fixed either 2 hours at room temperature or 4˚C overnight. Embryos were then transferred to 100% methanol and stored at -20˚C for no longer than 1 week. For embryos older than HH11 (Hamburger and Hamilton, 1951), heads was perforated with a pin to assist better wash and prevent trapping of colour in whole-mount *in-situ* hybridisation.

2.2 Whole-mount *in-situ* hybridisation

2.2.1 Transformation of DNA

DH5-α competent cells were defrosted on ice (from storage at -80◦C). ~1 µg of DNA was added to 50µl cells and mixed. Cells were incubate on ice for 30min and gently mixed constantly. Cells were then incubated at 37˚C for 5min then on ice for 2min. After that, 500µl SOC (Super Optimal broth with Catabolite repression) was added and cells were incubated at 37˚C for 1hour. Cells were then plated on LB (Lysogeny broth) plate and were incubated at 37˚C overnight. One single colony was picked and grown in 5ml LB at 37˚C shaker for about 6 hours. Transfer the LB with competent cells to 200ml LB medium with 1ml ampicillin. Grow the medium overnight at 37˚C shaker.

2.2.2 Preparation of DNA

DNA was prepared based on the protocol from Qiagen kit. The LB medium from 2.2 was centrifuged at 4˚C 4000rpm for 15min. Supernatant was poured off and the pellet was resuspended. The bacterial pellet was then lysed at room temperature for 5min and was neutralised in ice for 15min. After that, the solution was spun at 4˚C at 4000rpm for 40min. At the same time, a Qiagen-tip 500 column was equilibrated. After centrifuge, the supernatant was applied to the Qiagen column. The column with DNA binding was washed for two times. After that, DNA was eluted to a clean tube. 11ml of isopropanol was added to precipitate DNA. The solution was spun at 4˚C at 4000rpm for 75min. The supernatant was poured off and the DNA washed by 500µl 70% ethanol for two times. The supernatant was poured off without disturbing the pellet. The DNA pellet was air-dried at room temperature and was resuspended in TE in 1µg/µl concentration. DNA was stored -20˚C.

2.2.3 DNA digestion

DNA was cut in 20µl total volume with 5µg plasmid, 2µl *Bovine serum albumin* (BSA), 2µl required buffer and 2µl restriction enzyme in distilled water. The solution was incubated at 37˚C for about 4-6 hours. After cutting, the DNA was checked in 1% agarose gel to see whether it was cut successfully. 80µl of Phenol:Chloroform (Sigma) was then added to remove protein contaminants. The solution was vortexed vigorously and was spun in a centrifuge for 15mins. Then the top layer was taken using an Eppendorf pipette. 10µl 3M Na Acetate was added for every 100µl and 2.5x its volume of 100% ethanol was added as well. The solution was vortexed briefly and the digested DNA was precipitated at -20˚C overnight.

2.2.4 DNA transcription

Precipitated DNA was harvested by centrifugation at 4000rpm for 30min. The supernatant was removed and the DNA pellet was washed with 150µl 70% ethanol.

The solution was vortexed well and was spin for another 15min. Alcohol was taken off and the pellet was dried at room temperature completely. After that, the pellet was dissolved in 15 µl distilled water at room temperature. 3 µg of DNA was transcribed in a total volume of 30 µl and the following solutions was added in order: 6 µl 5x transcription buffer (Promega), 2 µl DIG-nucleotide mix (Promega), 3 µl 10X DTT (Promega), 1 µl RNAsin (Promega), and 2 µl polymeras. The solution was mixed well and DNA was transcribed at 37˚C for at least 3 hours. After incubation, 1 µl DNase (Promega) was added and the solution was incubated at 37˚C for 30min to remove template DNA. At the same time, the probe length was checked in 1% agarose gel. Then 8 µl EDTA was added to stop the DNAase and was diluted by 50 µl of distilled water. Then 10 µl 4M LiCl and 250 µl absolute ethanol were added. The solution was vortexed well and DNA was precipitated at -20˚C overnight.

2.2.5 Probe making for whole-mount *in-situ* hybridisation

The transcribed RNA was spun at 4˚C at 4000rpm for 30min and alcohol was taken off carefully. The pellet was washed by 300 µl 70% ethanol and vortexed very well. The pellet was spun for another 15min and alcohol was taken off. The pellet was dried completely at room temperature. Then it was dissolved in distilled water at 1mg/ml and was incubated at 37˚C for 15mins, then 65˚C for another 15mins. The solution was vortexed occasionally. The probe was denatured at 95˚C for more than 3min and was immediately cooled on ice for at least 5min. Finally the probe was transferred to 10x volume of hybridization buffer to store at -20˚C.

Table 2.1 shows all ISH probes used in this project.

Gene name	Provider/ Est humber	Size	Gene name	Provider/ Est number	Size
cNOT1	M. Kessel	~2000	PLK1S1	ChEST347j23	671
LMO7	ChEST860d14	1908	PIK3CD	ChEST886j23	516
GNPDA1	ChEST950h2	1241	PIK3R5	ChEST553J10	1083
KCNMA1	Paul Fuchs / Bernd Sokolowski	437	PPP1R14C	ChEST219i17	815
KIRREL3	ChEST883d8	593	SLC39A11	ChEST622b5	673
MCF2L	ChEST103i7	2802	ADRA2A	ChEST742a6	1113
PKIγ	ChEST318i8	1022	CHGA	ChEST139e12	742
ENPP4	ChEST686p14	627	NXPH1	G. Dechant	734
NRP1	G. Neufeld	~1000	FLBN7L	ChEST761l3	1212
NRSN1	ChEST129b19	1081	PCSK6	ChEST722i4	1361
PRKG1	ChEST206l15	674	PROM1	ChEST671b22	686
RAB11-FIP4	ChEST803n22	1579	SMOC1	ChEST66m21	831
SHH	In-house	~1000	TMEM63C	ChEST440l4	591
TMTC2	ChEST968a24	827	TSPAN6	ChEST407m3	469
VTN	E.Marti	~752	DLX5	In-house	315
DLX6	Andrea Streit	350	ID2	Marianne Bronner-Fraser	1239
LDB2	ChEST729m24	724	MSX1	In-house	332
MSX2	Monique Welten	331	BTG2	ChEST32k18	1998
DSP	ChEST293i19	756	MOXD1	Marianne Bronner-Fraser	~2300
SOCS2	ChEST586m16	870	CBLN2	ChEST718g2	689
CXCL12	Bearte Brand-Saberi	425	EFNA5	ChEST290o16	1404
PLXNC1	ChEST579j22	592	TNFRSF19	ChEST175o17	2060

2.2.5 Whole-mount *in-situ* hybridisation (Gall and Pardue, 1969)

Embryos in absolute methanol were rehydrated through 75%, 50% and 25% methanol in PTW (PBS with 0.1% Tween-20, Sigma). Then they was washed in PTW for 2 x10min. Proteinase K was then added for proteolytic digestion (1µg/ ml in PTW) and vials were carefully rotated to let the proteinase K rinse the whole bottle. EGK (Eyal-Giladi and Kochav) stages embryos (Eyal-Giladi and Kochav, 1976), were left in proteinase K for 5min. From stages up to HH12, embryos were left in proteinase K for 15min. For stages older than HH14, they were left in proteinase K for 30min at least. For stages older than 25, embryos were left in proteinase K for 1hour. Then proteinase K was taken off and embryos were rinsed carefully by PTW. Vials must be carefully rotated during this process to let PTW rinse the whole vial. Embryos were then post-fix to stabilize Glutaraldehyde was then added (0.1% in 4% paraformaldehyde) for 30min to post-fix and stabilize embryos. Embryos were then rinsed 2 times in PTW and 2 times in hybridization solution. Embryos were then incubated in hybridization solution in 70°C water bath for at least 2 hours. At this step, embryos can be stored in -20°C freezer in hybridization solution until required. Otherwise, they were heated in a 70°C water bath for at least another 2 hours before the required probe was added. Embryos were incubated with required probe in 70°C water bath overnight. On the second day, embryos were rinsed 3 times by hybridisation solution and then were incubated in hybridisation solution for 30min at 70°C for two times. Solution was replaced by 1:1 hybridisation solution: TBST and embryos were incubated in 70°C water bath for another 20min. Then embryos were washed 3 times by TBST and further 3 times for 30min in TBST at room temperature on the rocker. The blocking buffer was made as follow: 5% heat inactivated goat serum and 1mg/ml BSA in TBST. Embryos were blocked at room temperature for at least 2 hours. Blocking buffer was replaced by anti-dig antibody (1:5000 in block solution) and embryos were incubated at 4°C rocker overnight. On the third day, embryos were rinsed in TBST 3 times and were washed in TBST 3 times for 1 hour each at room temperature on the rocker. Embryos were washed at 4°C on rocker in TBST overnight. For old embryos (HH>20), more changes of TBST for washing were required. To develop colour of *in-situ,* incubated embryos in fresh-made NTMT (0.1M NaCl, 0.1M Tris

HCL(pH 9.5), 0.05M $MgCl_2$, 1% Tween-20 in distilled water) for 10min. Then embryos were incubated at room temperature in NTMT containing 4.5µl NBT and 3.5µl BCIP per 1.5ml. The vials were wrapped with film to protect from the light. The colour of embryos was checked every 15min. The reaction was stopped by washing 2 times in TBST and embryos were post-fixed in 4% paraformaldehyde. Pink or pale purple background can be washed out using absolute ethanol.

2.3 Histological section

Fixed embryos from whole-mount *in-situ* hybridisation were dehydrated in absolute methanol for 10min, then in propan-2-ol for 5min. Tetrahydronaphthalene was then added and the embryos were incubated at 60◦C for at least 30min. Then paraffin wax was added to tetrahydronaphthalene in 1:1 ratio and embryos were incubated at 60◦C for another hour. Embryos were solidified by clean wax at 60 ◦C for half a day and wax was changed several times. Embryos were poured into a mould and were allowed to set overnight. The embryos were sectioned at 5 ~ 10 microns using Microm HM315 Microtome. After sectioning, sections were dewaxed in histoclear for at least two times. Sections were mounted in Canada balsam.

2.4 Optical Projection Tomography (Sharpe, 2003)

2.4.1 Preparation of embryo

When embryos subjected to *in-situ* hybridisation are to be imaged by OPT, it is important to avoid developing the *in-situ* colour reaction for too long. This is controlled by diluting the chromogens for alkaline phosphatise 10 times as compared to the *in-situ* protocol outlined above (i.e. 4.5 µl NBT and 3.5 µl BCIP per 15 ml NTMT). Embryos stored in PFA were washed thoroughly in PBS for 48 hours.

2.4.2 Embedding embryo

1% solution of Low Melting Point Agarose (UltraPure LMP Agarose, invitrogen) was made up in distilled water. It was cooled to 60◦C and was filtered through Whatman filter paper 113V. The agarose was cooled down to 37◦C in incubator. A 50mm x 20.3mm (diameter x height) petri dish was prepared on top of an ice-cool platform. Embryos were transferred from PBS to the petri dish and PBS was removed as much as possible. The agarose was poured into the dish gently to avoid generation of bubbles. A glass pipette was used to gently move the embryo to the centre of the petri dish. Make sure when the gel sets, the embryo is still in the centre of the dish. After that, the embedding embryo was incubated in 4˚C fridge to further set for about 10 min.

2.4.3 Trimming the embryo

The agarose was trimmed to a rectangular block with the embryo in the centre of the block, leaving at least 5mm between the embryo and end of the block. Make sure the end of the block is completely straight. The surface of the block was dried and the end of the block was glued to the mount. Press gently to remove air bubbles and ensure complete contact between the block and the mount. The block was allowed to set for 5-10min. Use a blade to cut the four corners at the same angle. When cutting, start from the top of the block and cut outwards. So at the end, the block will have eight angled sides and an octagonal shape.

2.4.4 Dehydration and clearing of specimen

The mount was placed into absolute methanol and the lid had to be completely closed. Methanol was changed until no water remained in agarose block. Then methanol was replaced with BABB (2:1 Benzyl Benzoate: Benzyl Alcohol). The bottle was left with lids off to allow the remaining methanol to evaporate at room temperature and was protected from light. Embryos were scanned by OPT when the agarose block was transparent.

2.4.5 Scanning embryos

Before scanning embryos by OPT, alignment should be carried out. Alignment pin was used to check the rotation and maximise to largest magnification. Pin alignment was carried out in OPT scanning software. Once it was done, the mount was attached to the rotation stage and scanning could be started. Scanned images were reconstructed by NRecon Software.

2.5 Fate mapping

2.5.1 DiI and DiO preparation

DiI and DiO (Invitrogen) stock were dissolved (in dimethylformamide (DMF) at 2mM). Before labelling, 5µl stock DiI or 3µl stock DiO was added to 3µl 1:1000 Tween-20 in sucrose with 45 µl 0.39 M sucrose at 37˚C.

2.5.2 *in vivo* label

Eggs were placed horizontally and were incubated to required stages. A small window was opened on top of the eggs. Tyrodes was used to lift the embryos up above the egg shell. To visualise the embryos, ink (100 times diluted in tyrodes; winsor & newton black Indian ink) was injected underneath the embryos. Membranes on top of the embryos were peeled off. For stages older than HH14, the epidermis was carefully peeled off at the position that would be labelled. Dye was injected onto the cells at the desired location using a fine sharp calibrated pipette. The position of labelling was measured using 26mm crossed graticules (Pyser-SGI). Then about 2ml of egg albumen was removed to let the embryos sink down and 1 ml egg albumen was put back on top of the embryos. The window in the shell was then closed with PVC tape. Eggs were put back to 37◦C to incubate to the required stages.

2.5.3 Harvest labelled embryo

Dye labelled embryos were harvested as in 2.1. Labelled embryos were photographed before fixing in 4% paraformaldehyde.

2.6 Imaging

Fixed embryos were first transferred to PBS before imaging. Dyes labelled embryos were kept in tyrodes when imaging. The embryos were photographed using Olympus *SZH10* Zoom Stereo Microscope and QImaging camera (Retigar 2000R with QCapture Pro Software). After imaging, embryos were stored back in 4% paraformaldehyde fix.

2.7 Ablation of diencephalon

Embryos were incubated and prepared as in 2.5.2. The targeted tissues were removed using a microsurgical knife (5.0mm depth, Rincon). The embryos was covered with albumin as in 2.5.2 and the egg was taped back the window before incubating to the required stages. The embryos were harvested and fixed as in 2.1.

Chapter 3

Looking for putative organizers by studies of syn-expression groups

3 Looking for putative organizers by studies of syn-expression groups

3.1 Introduction

As discussed in chapter 1.5., 31 up-regulated and 16 down-regulated genes were selected based on the microarray assays and their expression patterns were used to find our putative organizers. As the microarray assays did not give spatial and temporal information, their expressions need to be mapped over the developmental period of interest. Whole-mount *in-situ* hybridisation was used. Embryos from pre-primitive streak EGK X-XIV (Eyal-Giladi and Kochav, 1976) to Hamburger and Hamilton (Hamburger and Hamilton, 1951) stage 14 were surveyed. The following chapter shows and discussed the expression patterns of syn-expressed genes in Table 1.3. The work of these whole-mount *in-situ* hybridisation was done in co-operation with Dr. Anderson and the full expression patterns for each gene is shown in Appendix 1.

3.2 Results

3.2.1. Expression of distilled list genes in known organizers

The microarray assays described in chapter 1.5 selected genes that are expressed in early and/or late Hensen's node. As it is shown in Figure 3.1, there are 25 out of 31 distilled list genes expressed in Hensen's node in whole-mount *in-situ* bridisation compared the microarray assays. In comparison, expressions of *PIK3CD*, *NXPH1*, *NRSN1*, *PROM1*, *TMTC2* and ChEST125i5 were not seen (data in Appendix 1). It may be the hybridisation experiment was not as sensitive as the microarray assay. But most of the organizer-enriched genes in the distilled list were detected, which confirms that the microarray assays had selected genes of interest correctly. In addition, all of the organizer-depleted genes selected from the microarray assays were not detected in Hensen's node from HH3+ to HH6 (data in Appendix 1).

Figure 3.2 shows the expression patterns of these organizer-depleted genes at HH4. *MSX1* is the best example showing that the organizer-depleted gene is expressed in the posterior, non-organizer part of the embryo, but not in the node.

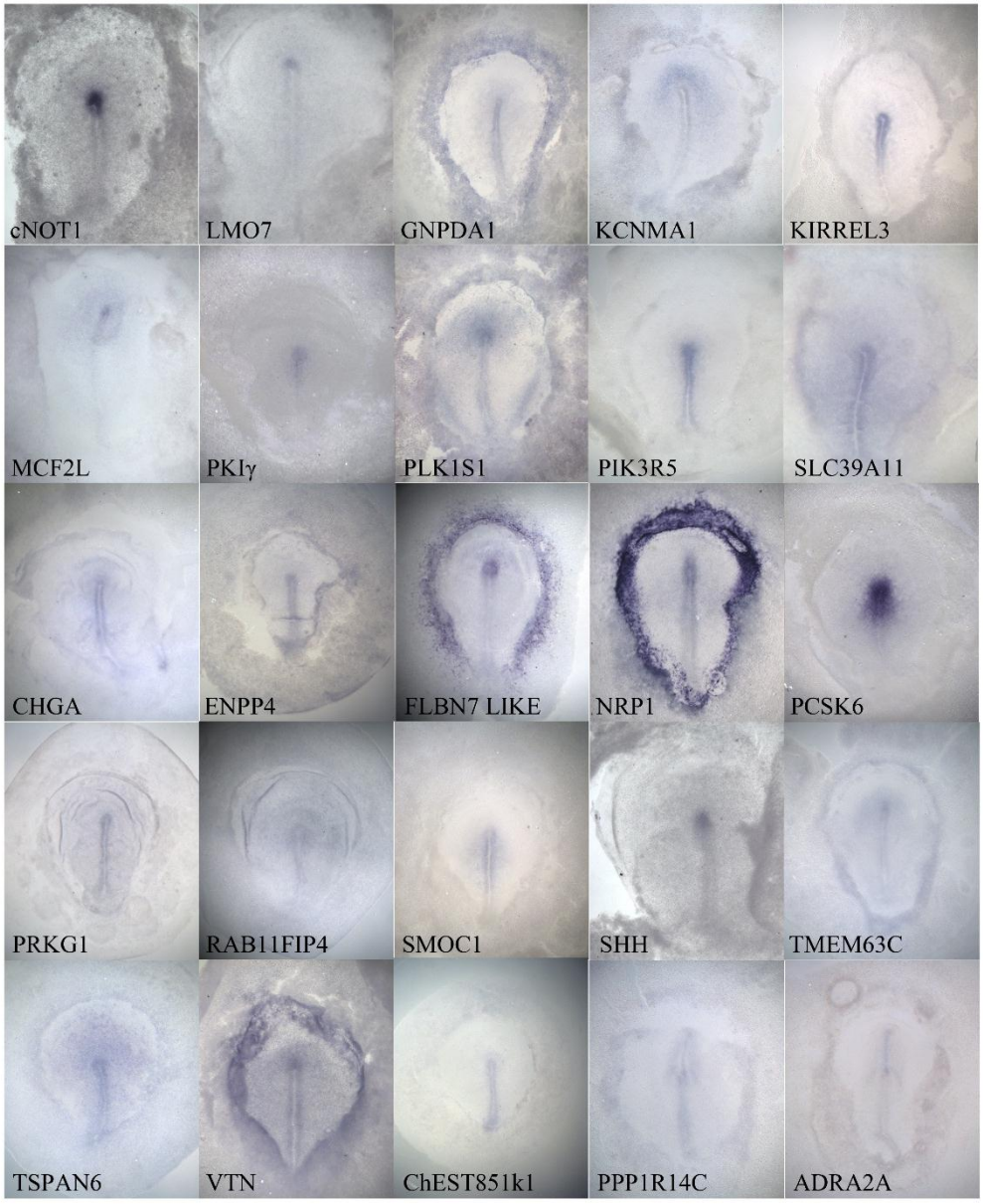


Figure 3.1 Expressions of organizer-enriched genes in the Hensen's node. All these genes are expressed at HH4, except *PPP1R14C* and *ADRA2A* which were expressed lately at HH6. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

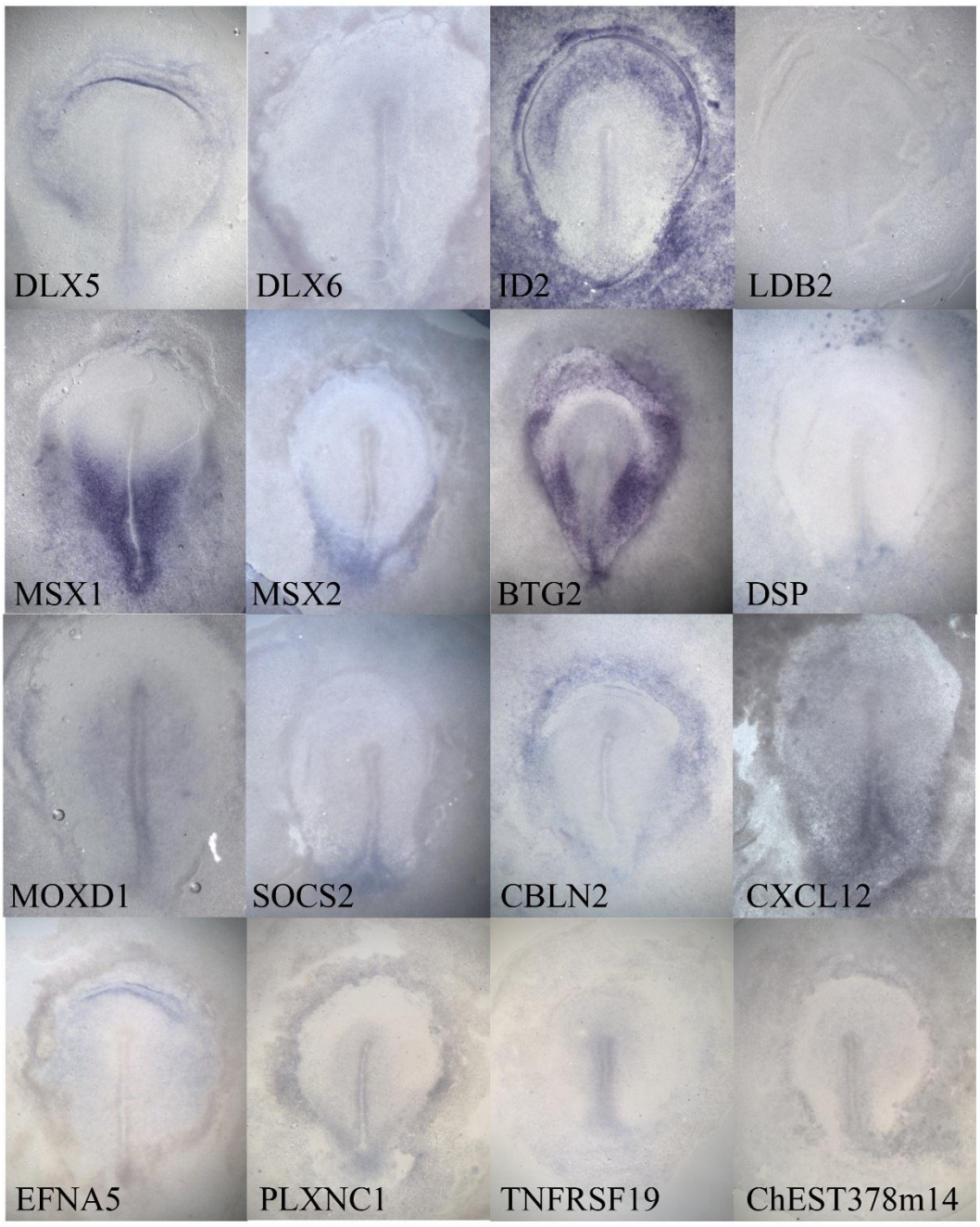


Figure 3.2 Expressions of organizer-depleted genes in the Hensen's node. All these genes were not expressed in Hensen's node at HH4. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

Apart from Hensen's node, another organizer that was analysed in the microarray assays was the notochord and floor plate. Figure 3.3 shows the expression patterns of 30 organizer-enriched genes between HH11 and HH12. Their expressions in notochord at these stages were commonly strong, although the length of

expressions during development was different for each gene (data in Appendix 1). There was only one exception is *PROM1*, which had poor expression in the notochord and might be due to a probe that is difficult to detect. On the other hand, all 16 organizer-depleted genes were not detected in the notochord between HH11 and HH12. *MSX1* was again the best example showing that it was expressed in the non-organizer, dorsal neural tube but not in the ventral part.

The final organizer that was analysed in the microarray assays was the ZPA and the whole-mount *in-situ* hybridisation for embryos at HH22 or later was done by Prof. Burt group. Combining their data and the expression patterns discussed above, it could be confirmed that the microarray assays were successful in choosing genes enriched or depleted in current known organizers. To find out potential new organizers, the expression patterns of all these genes were analysed by whole-mount *in-situ* hybridisation first and our group was mainly focused to study the development from EGK stages to HH14. In the next chapter, I am going to discuss the expression patterns of regions that these syn-expressed genes expressed in common.

Figure 3.3 Expressions of organizer-enriched genes in the notochord. All these genes were expressed in the notochord between HH11 and HH12. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

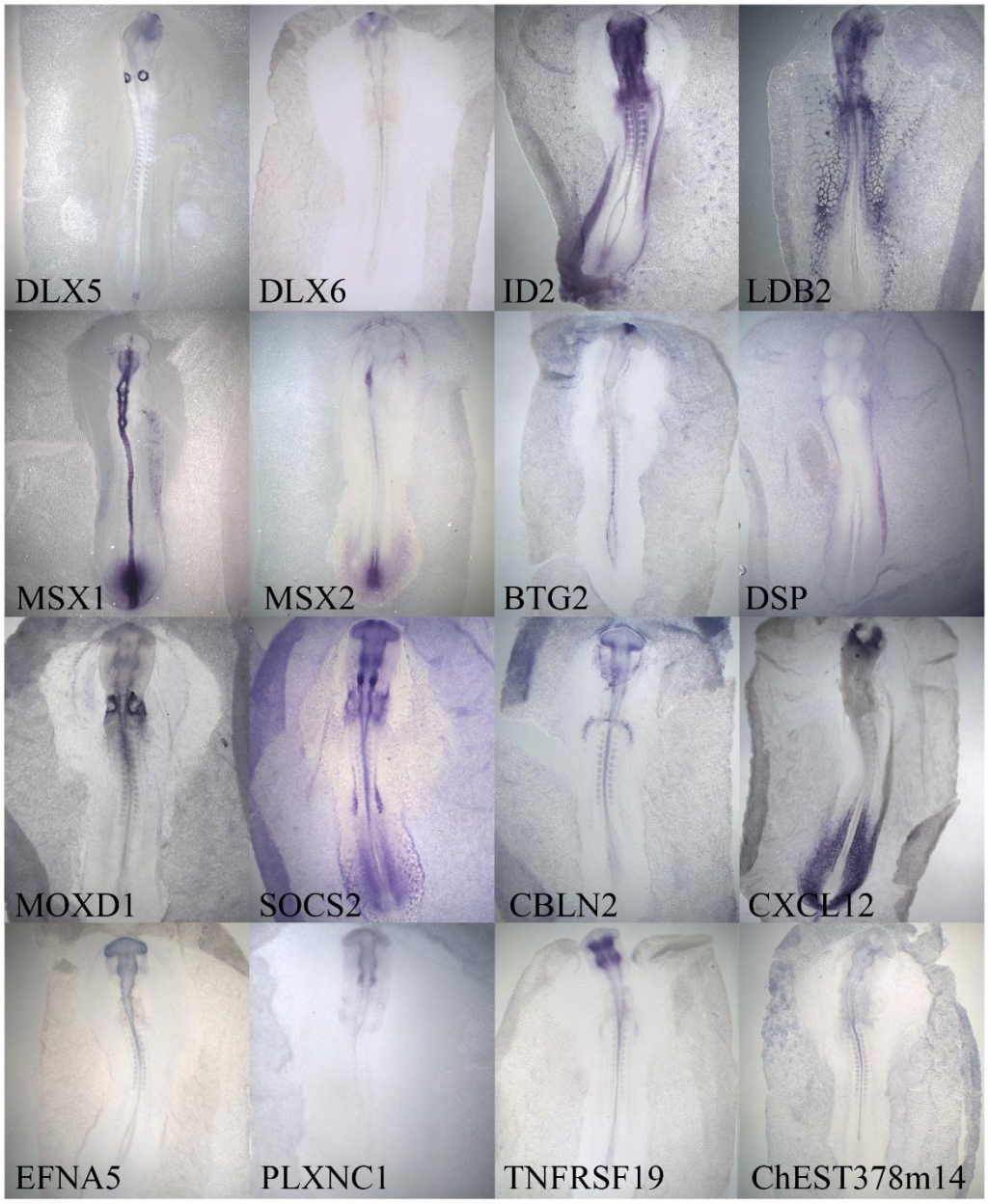


Figure 3.4. Expressions of organizer-depleted genes in the notochord. All these genes were not expressed in the notochord between HH11 and HH12. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

3.2.2 Summary of regions that syn-expressed genes express in common

Region	Organizer-enriched genes expressed	Organizer-depleted genes not expressed
Otic vesicle	GNPDA1, KIRREL3, MCF2L,PLK1S1, PIK3R5, ADRA2A, ENPP4, SMOC1, TSPAN6, ChEST125i5	LDB2, MSX1, MSX2, BTG2, SOCS2, CBLN2, CXCL12, EFNA5, PLXNC1,TNFRSF19, ChEST378m14
Pharyngeal arches	LMO7, GNPDA1, PLK1S1, PIK3R5	LDB2, BTG2, DSP, SOCS2, CXCL12, EFNA5, TNFRSF19, ChEST378m14
Somites	KIRREL3, MCF2L, PKIγ, ENPP4, NRP1, PCSK6, PRKG1, RAB11-FIP4, SMOC1, TMTC2	DLX5, DLX6, MSX1, MSX2, DPS1, SOCS2, CXCL12, EFNA5, PLXNC1, TNFRSF19, ChEST378m14
Anterior intestinal portal endoderm	FBLN7L, NRP1, NRSN1, SHH, TMTC2, VTN	DLX5, DLX6, LDB2, MSX1, MSX2, MOXD1, SOCS2, CXCL12, EFNA5, PLXNC1, ChEST378m14
Lateral plate mesoderm	KIRREL3, PKIγ, NRP1, SMOC1	DLX5, DLX6, LDB2, MSX1, MSX2, BTG2,SOCS2, CBLN2, EFNA5, PLXNC1, TNFRSF19, ChEST378m14
Pineal gland	cNOT1, TSPAN6, PKIγ	DLX5,DLX6,MSX1,MSX2, LDB2,SOCS2,DSP,BTG2, PLXNC1,CXCL12,CBLN2,ChEST378m14

Table 3.1 Summary of gene expression patterns in the regions of potential organizers, having organizer-enriched genes commonly expressed, and organizer-depleted genes that are not expressed.

As it is shown in Table 3.1, there were several regions that had organizer-enriched genes expressing, including the otic vesicles (Figure 3.5), pharyngeal arches (Figure 3.6), somites (Figure 3.7 and 3.8), the endoderm of anterior intestinal portal (AIP) (Figure 3.9), lateral plate mesoderm (Figure 3.10) and pineal gland (Figure 3.11). Whether these regions have organizer abilities has not been studied before. In all of these regions, most of the organizer-depleted genes were not expressed. The region with the least expression was the pharyngeal arches, which only had half of the organizer-depleted genes down-regulated in its region. On the other hand, the organizer-enriched genes were expressed differently in these potential organizing regions. Compared to known organizers described in chapter 3.2.1, all of these regions have fewer than half of the organizer-enriched genes expressed and most of these genes are not transcription factors. The pharyngeal arches and pineal gland were the only two that had organizer-enriched transcription factors expressed: *LMO7* was expressed in the pharyngeal arches, while *cNOT1* was expressed in the pineal gland. In addition, the otic vesicles and somites had nearly 10 organizer-enriched genes expressed, but the area of expression was different for each gene. In the otic vesicles, *GNPDA1* and *TSPAN6* were expressed more anteriorly, while *MCF2L* and *PIK3R5* were expressed posteriorly. On the other hand, genes expressed in the somites could be divided into three different types. *KIRREL3*, *PCSK6*, *PRKG1* and *SMOC1* were expressed in posterior newly formed somites. *MCF2L*, *ENPP4* and *TMTC2* were expressed in anterior somites. *PKIγ*, *RAB11-FIP4* and *NRP1* could be seen in all somites along the embryo. Lateral plate mesoderm had only 4 organizer-enriched genes expressed, and these genes were also expressed in differently. *SMOC1* was expressed posteriorly compared to the rest that were expressed in anterior lateral plate mesoderm. Finally, the endoderm of anterior intestinal portal (AIP) had 6 organizer-enriched genes expressed and their expression patterns were similar, as shown in Figure 3.9. Dr. Anderson chose to further study the organizing ability of AIP.

For the rest of my project, I selected the pineal gland as a region of interest. The first reason was it is one of the common sites that express organizer-enriched transcription factor. Unlike secreted molecules which can be seen in broader area around the organizer tissue, transcription factor is easier to detect in local

organizing region. Also, compared to the pharyngeal arches, which also expressed transcription factor, the pineal gland had more organizer-depleted genes that were not expressed within it. In the following chapter, I will further discuss the expression patterns in pineal gland.

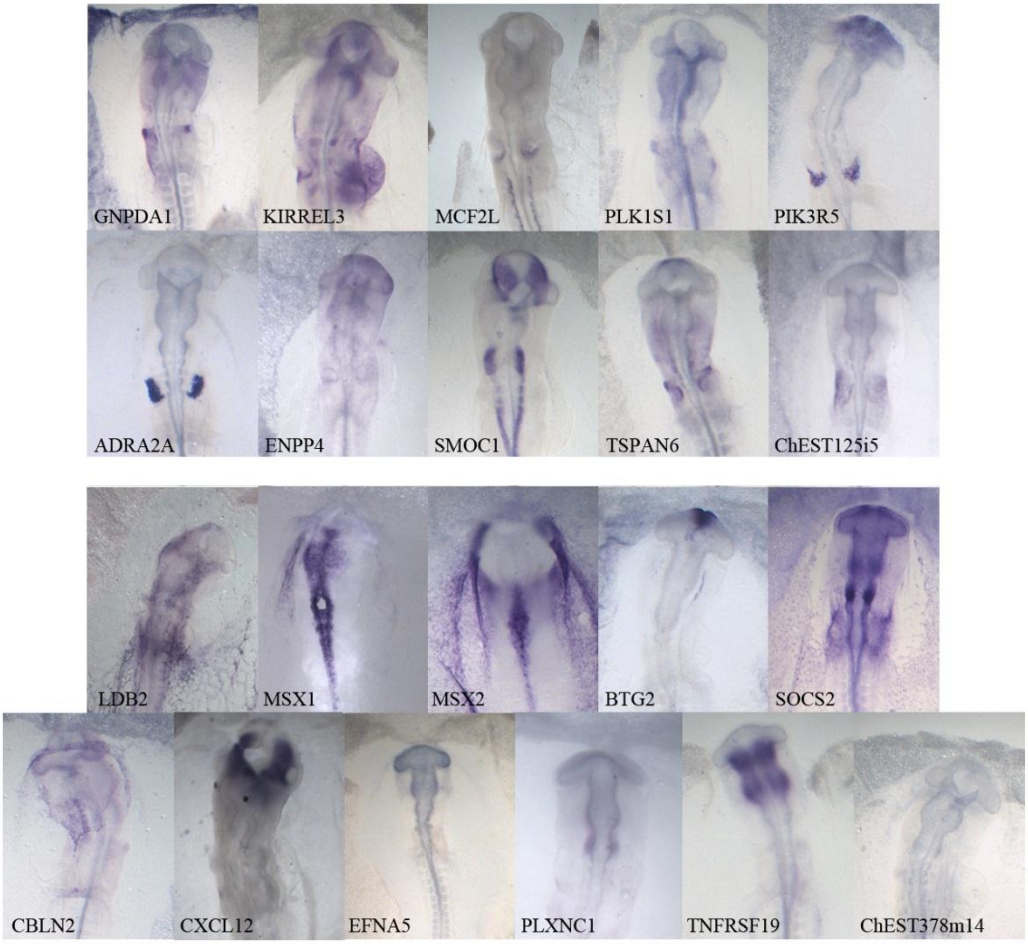


Figure 3.5. Expressions of distilled list genes in otic vesicles. The first two rows show the expressions of organizer-enriched genes expressed in the otic vesicles, while the bottom two rows show the organizer-depleted genes that were not expressed in the otic vesicles. Embryos selected were between HH10 and HH12. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

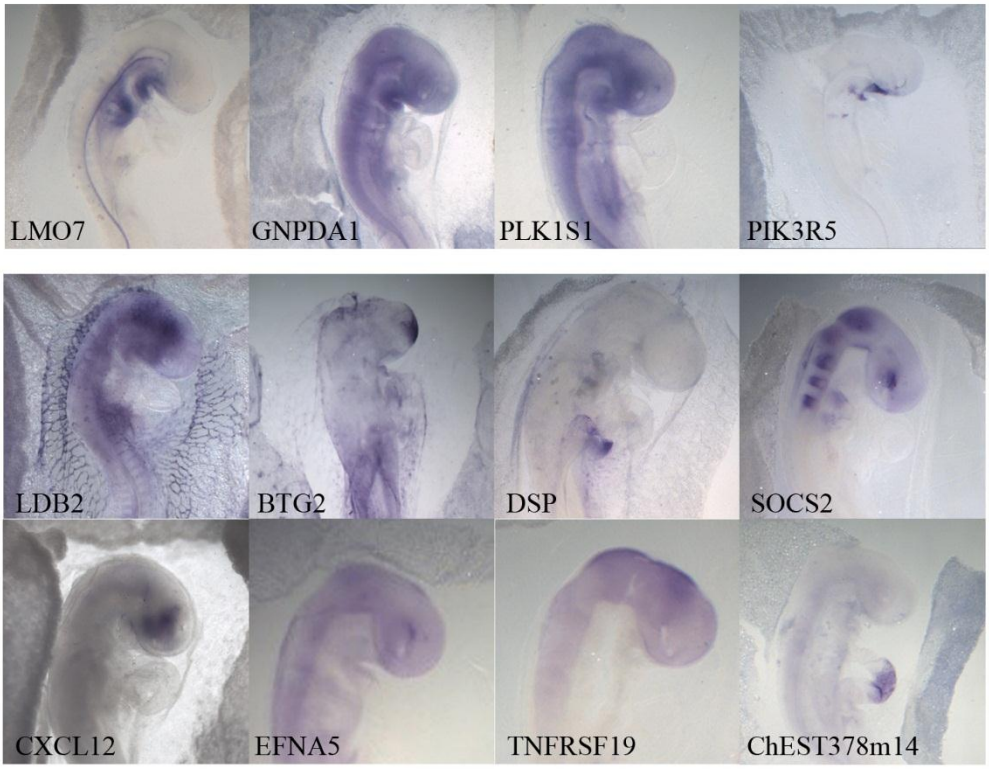


Figure 3.6. Expressions of distilled list genes in pharyngeal arches. The first row shows the expressions of organizer-enriched genes expressed in the pharyngeal arches, while the bottom two rows show the organizer-depleted genes that were not expressed in the pharyngeal arches. Embryos selected were at HH14. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

Figure 3.7 Expressions of organizer-enriched genes in somites. Embryos selected are at HH14. The first row of genes were expressed in the newly formed somites, while the second row of genes were expressed in the somites of anteiror side. The bottome row shows genes that are expressed in all somites. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

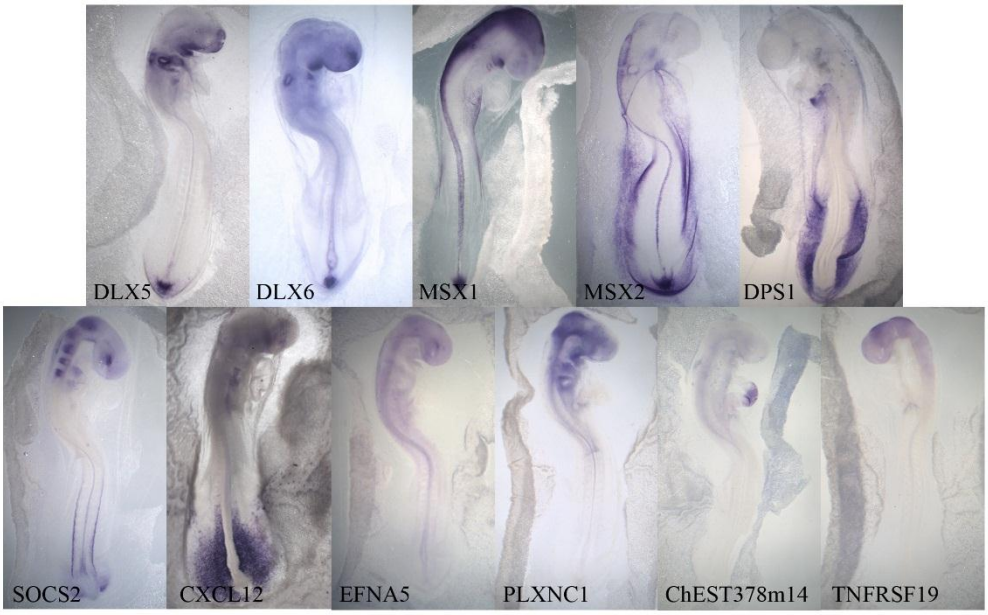


Figure 3.8 Expressions of organizer-depleted genes in somites. Embryos selected were at HH14. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

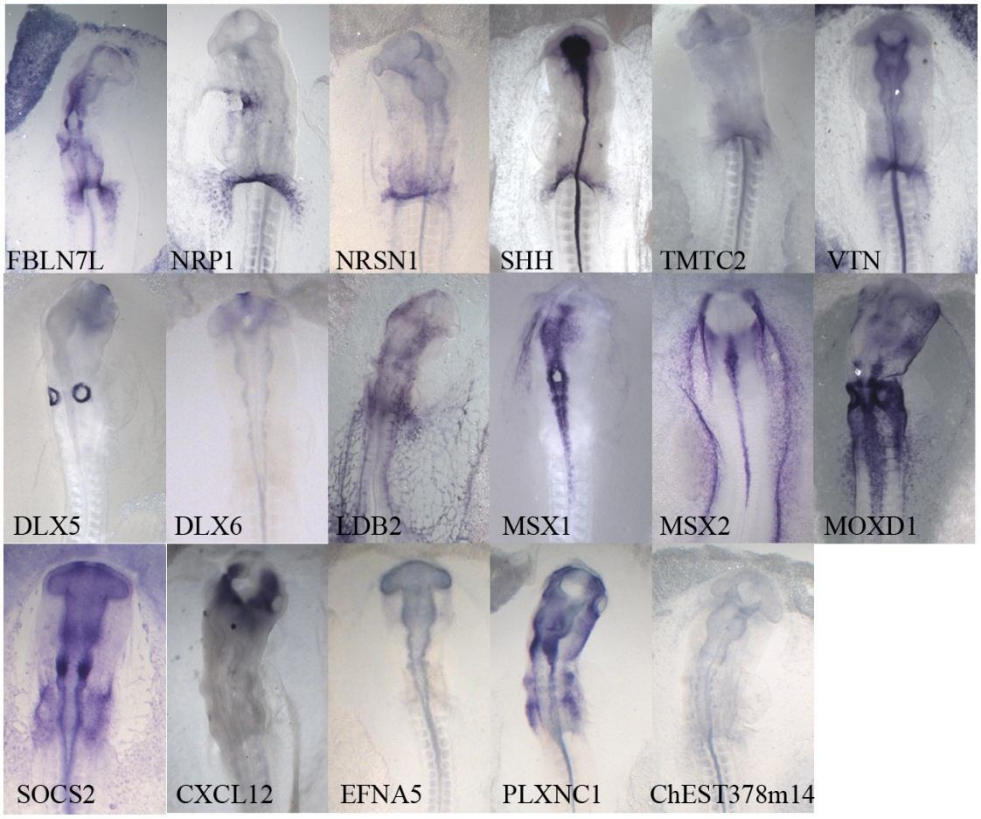


Figure 3.9 Expressions of distilled list genes in the endoderm of anteiror intestinal portal (AIP). Embryos selected were between HH10 and HH12. The first row shows the expression patterns of organizer-enriched genes in AIP, while the bottom two rows show the expression patterns of organizer-depleted genes. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

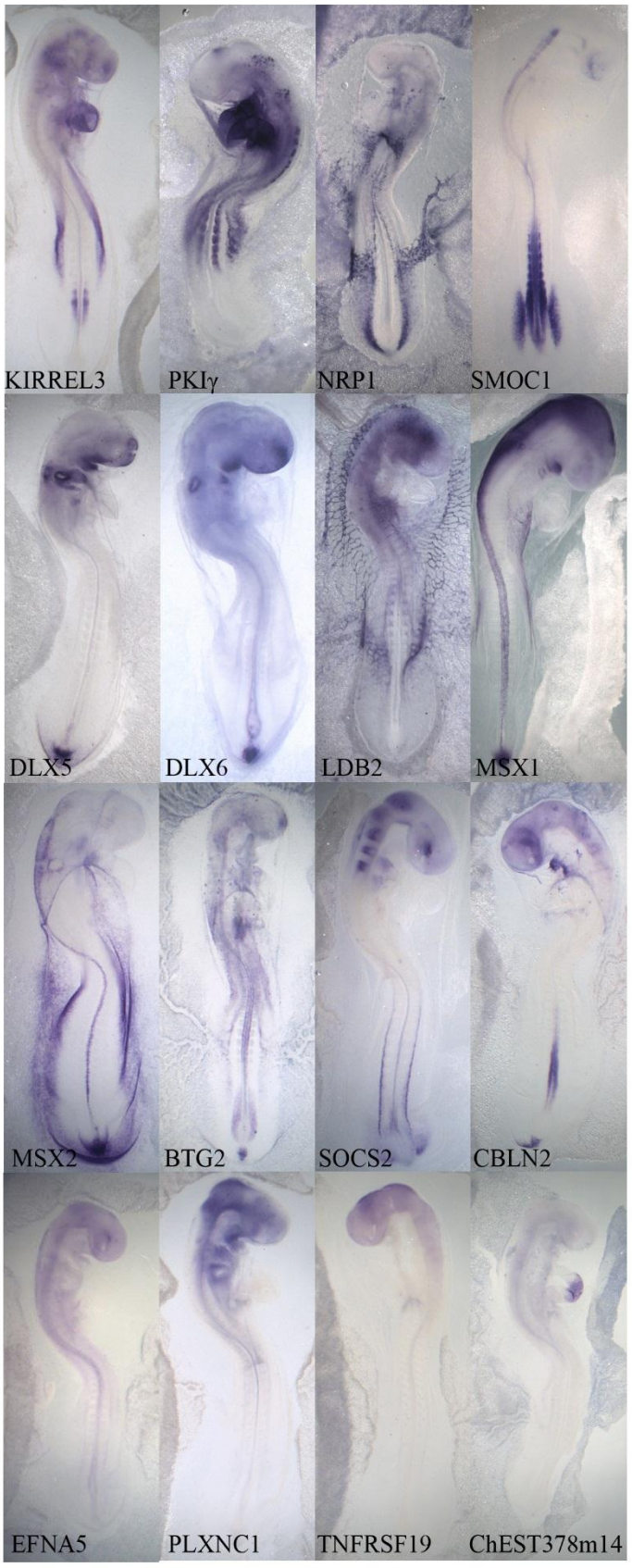


Figure 3.10 Expressions of distilled list genes in the lateral plate mesoderm. Embryos selected were at HH14. The first row shows the expression patterns of organizer-enriched genes in the lateral plate mesoderm, while the bottom three rows show the expression patterns of organizer-depleted genes. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

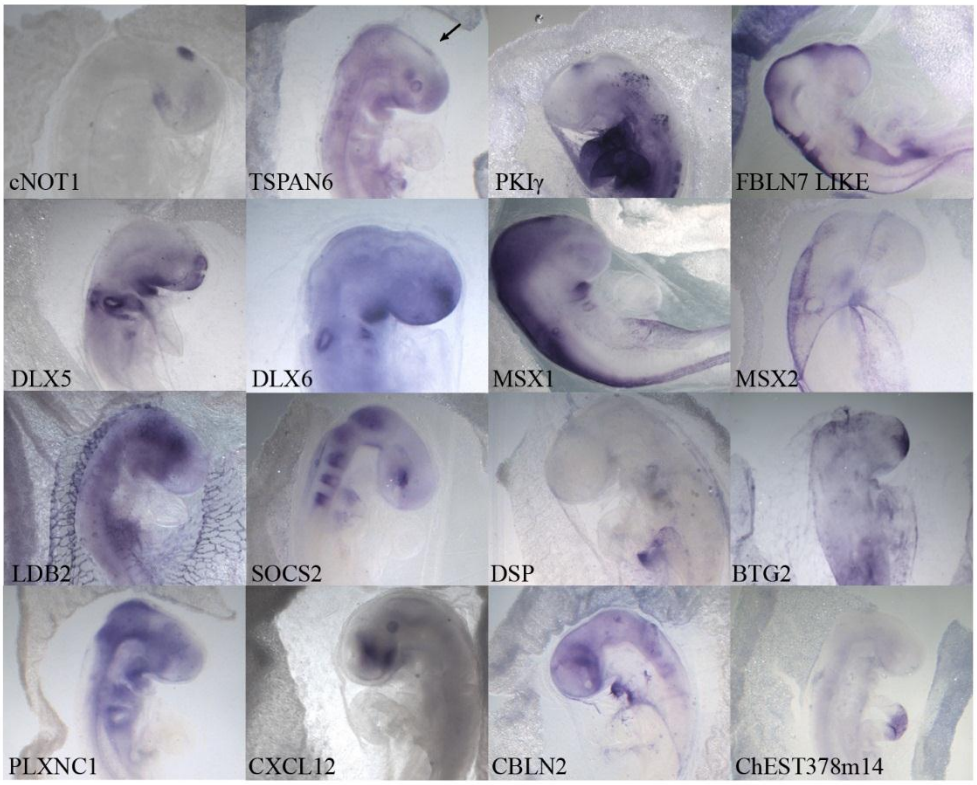


Figure 3.11 Expressions of distilled list genes in the pineal gland. Embryos selected are at HH14. The first row shows the expression patterns of organizer-enriched genes in the pineal gland. *FBLN7 LIKE* was not expressed in the pineal gland, but around the pineal gland. On the other hand, the bottom three rows show the expression patterns of organizer-depleted genes. These whole-mount *in-situ* hybridisations were done in co-operation with Dr. Anderson.

3.2.3 Pineal region is one of the potential organizer regions

The region that I wanted to further study was the roof of dorsal thalamus, which develops into the pineal gland in later stages. As it is shown in figure 3.11, there was one out of the two transcription factors enriched in known organisers that was also expressed in the pineal region, *cNOT1*. An intracellular protein, *PKIγ* and a transmembrane protein, *TSPAN6* were detected in the pineal region. If the roof of the diencephalon is a potential organizer, transcription factors, intracellular molecules and transmembrane proteins are expressed within organizing regions. In

comparison, secreted molecules may be detected in surrounding tissues adjacent to organizers. In Figure 3.11, *FBLN7L* mRNA was excluded from the pineal region and was observed in the tissues surrounding the roof of the diencephalon. As it is an extracellular matrix protein, it cannot be excluded that it may be involved in potential organizing activities as a matricellular proteins that interacts with other signalling molecules and is involved in cell-cell interaction. On the other hand, Figure 3.11 shows expressions of all syn-expressed genes that are depleted in organizers and their expression in the diencephalon at HH14. Five out of six down-regulated transcription factors were not expressed in the pineal region, *ID2* is the exception. Also, three out of four intracellular molecules were not expressed in pineal region except *MOXD1*. Four out of five membrane-bound or secreted molecules were not expressed in pineal region except *TNFRSF19* and *EFNA5*. And finally, the unannotated gene, *ChEST378m14* was not detected in the pineal region.

In conclusion, there was a small proportion of organizer enriched syn-expressed genes present in pineal region. But one of the two transcription factor was expressed, which is important to define organizing regions. On the other hand, only a few organizer depleted genes were expressed in pineal region. Therefore, pineal region is one of the potential organizer region to study further.

3.3 Discussion

The above chapter discussed those putative organizer regions. Traditional transplantation experiments required tests of large amount of pieces of tissues and an ectopic responding region. In comparison, this project aims to find out organizer-specific genes, which are either enriched or depleted in known organizers including Hensen's node, the notochord and floor plate and the ZPA. Microarray assays were performed to compare the gene expression levels between organizer and its closely related non-organizing regions (Figure1.5). There are many genes up or down-regulated in an organizer at particular stage. However, if cross comparisons are made between different groups as in Table 1.2, the number of genes enriched or depleted in early and/or late node, and ventral neural tube and

posterior limb bud (ZPA) remarkably few genes are found to be expressed in common; a total of 31 up-regulated genes and 16 down-regulated genes (Table 1.3). These putative syn-expressed genes include transcription factors, intracellular molecules membrane and secreted molecules.

Some of them are known to be involved in developmental process. For example, *cNOT1* is a transcription factor expressed in Hensen's node and notochord. In zebrafish, mutation in *cNOT1* homolog floating head (*flh*) causes an absence of *ntl* (mouse *Brachyury* homolog) expression in notochord precursors and leads to the lack of notochord in embryos. Hence, *cNOT1* is considered to be involved in notochord development in zebrafish (Talbot et al., 1995). Similar function in notochord formation of *flh* homolog *Xnot* in *Xenopus*, where overexpression of *Xnot* can mimic the effect of BMP inhibition and promote the formation of ectopic notochord (Yasuo and Lemaire, 2001). In addition, mouse *Noto* gene is shown to be involved in the regulation of node morphogenesis and left-right asymmetry as mutant embryos have varied size and shape of posterior notochord, shortened cilia and randomised left-right internal organs (Beckers et al., 2007). In comparison to transcription factors which regulate the expression of down-stream molecules in organizing activity, secreted molecules are released in organizing region and instruct neighbouring cells. One example is *SHH*, which was also selected in our microarray assay. In limb development, it is released by the ZPA in vertebrate and mediates the polarizing activity. As a morphogen, it diffuses across the posterior-anterior axis of the limb bud (Chang et al., 1994; Lopez-Martinez et al., 1995; Riddle et al., 1993a). In addition, signalling pathways that have been mentioned in Chapter 1 like Wnt signalling involve interactions with intracellular molecules. β-catenin is one sample intracellular molecule that interferes Wnt pathway and gene ablation of β-catenin leads to inactivation of Wnt signalling and thus abnormal structures in posterior midbrain and cerebellum (Brault et al., 2001; Chilov et al., 2010). The selection of genes involved transcription factors, membrane, intracellular and secreted molecules will help us to understand more about the characteristic of organizers and the mechanisms of organizing activities. Furthermore, many of the genes selected from our microarray assay have unknown functions in chick embryo development. However, some of them have been proved to be involved in development in other animal model, for example, *ADRA2A*, which

affects the development of cortical circuits in the mouse (Riccio et al., 2012). Also, some molecules are involved in important signalling pathway, for example, *PPP1R14C*, which interacts with ERK signalling (Wenzel et al., 2007).

As long as syn-expressed genes were selected, common sites of expression of these genes were going to be analysed, which require mapping of gene expression along the development of embryo. Whole-mount *in-situ* hybridisation was performed for chick embryos from EGK stages and up to HH14. This detail analysis of gene expression gives us more temporal information of expression of each gene. Also, *SHH* and *MSX1* were selected as controls once microarray assays had been done as they are known to be involved in organizing activities as discussed before. The expressions of *SHH* and *MSX1* correlated to the microarray data, demonstrating that the microarray analysis was successful. Although some of the genes were expressed weakly during the development, it may be due to the high sensitivity of the microarray. At the end, syn-expressed genes including *cNOT1*, *TSPAN6* and *PKIγ* were found to be expressed in the centre of the roof of the dorsal thalamus. *FBLN7L* which is a secreted molecule was expressed around the centre of the roof of the dorsal thalamus. Although, there were only 3 of 31 organizer-specific enriched genes expressed in the dorsal thalamus, it was still an interesting region to study. To compare, not all organizer-enriched genes from the distilled list is expressed in the ANR, which is another known organizer. For example, *PLK1S1* is not expressed in ANR up to HH14 (data in Appendix 1). This may due to the differences between each organizer. In addition, *cNOT1*, which is one of the two organizer-enriched transcription factors, was expressed in the roof of the dorsal thalamus. As discuss before, transcription factor is more likely to be expressed within the organizing region, and thus more likely to characterize the organizer. As it is shown in Figure 3.2 and in Kessel Mackem papers, *cNOT1* is expressed in very few regions including Hensen's node, the notochord, posterior digital limb and the roof of dorsal thalamus (Stein et al., 1996). Two of them are known organizers. Furthermore, in the zebrafish model, mutation in *flh* causes a premature termination of pineal cells differentiation but does not affect the development of parapineal, which means *flh* is involved in development of pineal (Snelson et al., 2008). Therefore, *cNOT1* may be involved in the development of the pineal gland in chick

embryo as well due to the possibility of evolutionary conservation. Secondly, there is a large proportion of organizer-depleted genes that were not expressed in the dorsal thalamus. Finally, although *FBLN7L* was not expressed in the centre of the roof of the dorsal thalamus, it was expressed in the surrounding regions. As *FBLN7L* is a secreted molecule, the possibility that *FBLN7L* may be involved in the organizing activities through interactions with extracellular proteins and surrounding cells cannot be excluded.

Furthermore, genes that require further analysis have been processed to histological section or optical projection tomograph. All this data is uploaded to www.echickatlas.org, which contribute to a new atlas of gene expression of chick embryo.

In conclusion, comparison of gene expressions between organizers and closely related non-organizing regions identified a set of syn-expressed genes that are enriched or depleted in known organizers. This set of genes involves different types of molecules including transcription factors and secreted molecules which play different roles in organizing activities. By studying their gene expression patterns over early chick embryo development, the centre of the roof of the dorsal thalamus was identified to have some common expressions of syn-expressed organizer enriched genes and lack of expressions of majority of organizer depleted genes. Therefore, it was considered to be a potential organizer.

Chapter 4

Development of early diencephalon in chick embryo

4 Development of early diencephalon in chick embryo

4.1 Introduction

In the development of the embryonic brain, there are two secondary organizers found so far, the anterior neural ridge (ANR) and the isthmus (chapter 1.3). The ANR is located at the most anterior part of neural plate and patterns the anterior-posterior axis of the telencephalon (Crossley et al., 2001; Houart et al., 1998b; Shimamura and Rubenstein, 1997a; Wilson and Rubenstein, 2000). In comparison, the isthmus functions as an organizer between mesencephalon and metencephalon and patterns the anterior hindbrain and midbrain (Alvarado-Mallart, 1993; Joyner, 1996; Marin and Puelles, 1994; Wilson and Rubenstein, 2000; Wurst and Bally-Cuif, 2001). Diencephalon is derived from the prosencephalon and locates posterior to the telencephalon. Many studies consider that the zone limitans intrathalamica (ZLI) as an organizer patterning the development of the diencephalon. ZLI is a transverse ridge of neuralepithelium located in the middle of diencephalon. It is also a boundary separate the dorsal and ventral thalamus. (Nieuwenhuys, 1999; Puelles et al., 1987; Rubenstein et al., 1994) *SHH* expresses in ZLI and is considered to be involved in patterning the ventral-dorsal axis of the diencephalon (Hashimoto-Torii et al., 2003; Kiecker and Lumsden, 2004; Vieira et al., 2005) . It is shown by quail-chick ectopic transplantation that *SHH*-expressing cells induced the expression of *Gbx2*, which is a marker for the dorsal thalamus (Echevarria et al., 2003). Similarly, the increased expression of *SHH* in thalamic progenitors induces rosto-ventral markers in mice, while reduction of *SHH* leads to expansion of caudal-dorsal markers (Vue et al., 2009). Bead experiments in zebrafish confirm the conserved role of *SHH* in patterning the thalamus (Scholpp et al., 2006; Zeltser, 2005). Hence, expression of *SHH* in the ZLI is required for normal development of the prethalamus and thalamus. However, there are no transplantation experiments showing that the ZLI alters the fate of cells in other regions, which argues if it is a true organizer (based on criteria in chapter 1) or a signalling centre.

As the diencephalon develops, it is subdivided into the dorsal and ventral thalamus, epithalamus and pretectum. Each compartment is separated by ridges and furrows

in the diencephalon (Figdor and Stern, 1993; Larsen et al., 2001). The epithalamus comprises of the habenula, and the pineal gland. The habenula locates in the bilateral thalamus and connects to the ventral midbrain and hindbrain through the stria medullaris (Sutherland, 1982; Villalon et al., 2012). It exhibits a left-right asymmetry in many animals including the fish, frog and chick (Concha and Wilson, 2001). On the other hand, the pineal gland locates at the centre of the dorsal epithalamus. It connects to the epithlamus by a stalk structure called pineal recess (Calvo and Boya, 1978; Calvo and Boya, 1979).It regulates the circadian cycle through the secretion of melatonin (Csernus et al., 2007). In fish, reptiles and amphibians, there is an extra pineal body, usually on the left hand side, called parapineal. The parapineal is involved in the asymmetric development of the diencephalon (Gamse et al., 2003).

In zebrafish, a homeobox gene *floating head* expresses in the pineal gland and is required for its normal development (Snelson et al., 2008). It is also found to be expressed in the dorsal telencephalon, the roof of prosomere 3 and the epithalamus (Staudt and Houart, 2007). *flh* is also involved in the regulation of development of the notochord in zebrafish (Talbot et al., 1995). From chapter 3, it is shown that *cNOT1*, which is the homolog of zebrafish *flh*, expresses in dorsal thalamus in HH14. At that stage, the epithelium of the dorsal thalamus is undifferentiated (Calvo and Boya, 1978). We first questioned how *cNOT1* expression relates to the development of the pineal gland. Also, we questioned whether the development of the pineal gland involves the movement or the regulation of the *cNOT1*-expressing cells.

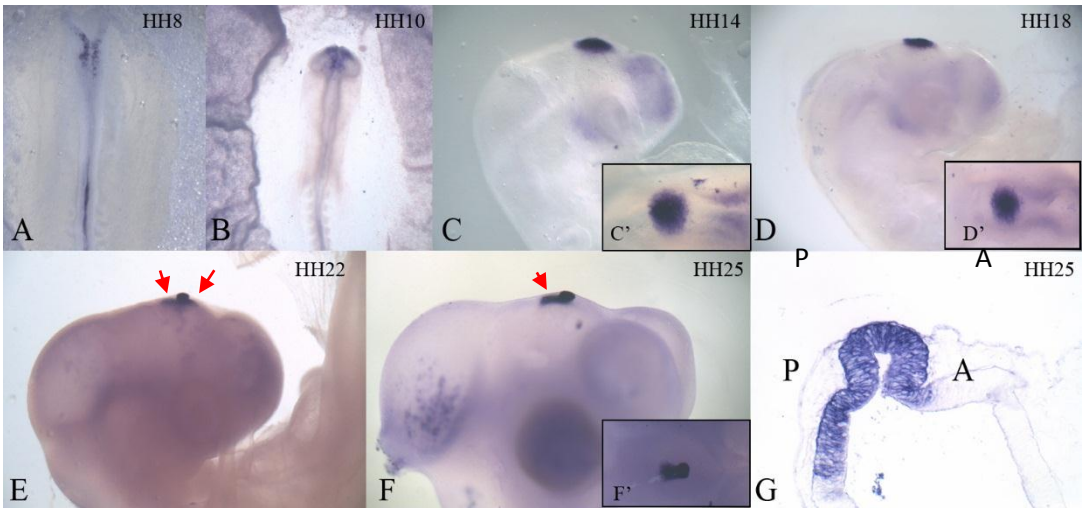
4.2 Results

4.2.1 *cNOT1* expression during diencephalon development

To study the role of *cNOT1* in the development of pineal gland in chick embryo and to compare that to the zebrafish, the expression of *cNOT1* was analysed in the developing diencephalon from HH8 to HH24. Whole-mount *in-situ* hybridisation

was done as it is described in Chapter 2 and *cNOT1* cDNA used was the same as in Chapter 3.

The expression of *cNOT1* was first observed in the dorsal neural tube at HH8 (Fig 4.1.A). At HH10, *cNOT1* expresses both in the telencephalon and diencephalon regions (Fig 4.1.B). At HH14, expression of *cNOT1* became restricted to the centre of the roof of the dorsal thalamus (Figure 4.1 C). The lateral borders of *cNOT1* expression reaches the lateral ridges seen on the roof of the dorsal thalamus (Figure 4.1 C'). The expression of *cNOT1* at HH14 embryo was also confirmed by the OPT scan, which gives more information in 3D (Figure 4.1 H). This broad expression in the dorsal thalamus was observed at HH18 as well (Figure 4.1 D and D'). The pineal gland starts to become morphologically visible as an obvious protrusion from about HH22. Expression of *cNOT1* at this stage became restricted to the pineal gland and to a small region in the surrounding epithelium (Figure 4.1 E). Based on other literature, the epithelium of the pineal gland was observed to be thicker than the surrounding tissues at HH25, although no pinealocytes can be distinguished yet (Calvo and Boya, 1978). *cNOT1* expression at HH25 was restricted to the pineal gland and the posterior midline of dorsal thalamus but not in anterior tissues (Figure 4.1 F, F' & G).



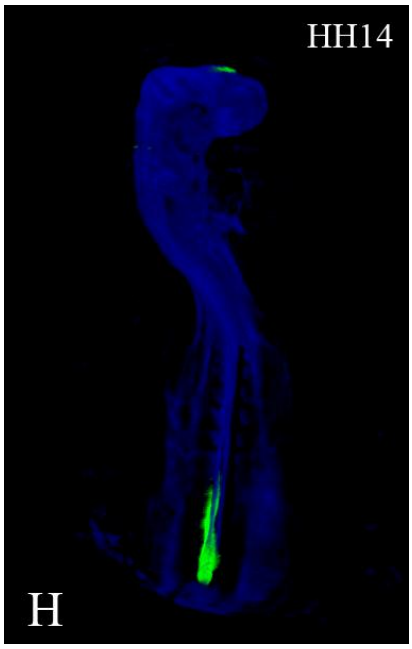


Figure 4.1 *cNOT1* expression in the developing diencephalon. Whole-mount *in-situ* hybridisation of *cNOT1* expressions in chick embryos are shown from A-F. *cNOT1* started to be expressed in the dorsal neural tube from HH8 (A, Data kindly provided by Dr. Claire Anderson). At HH10 *cNOT1* was expressed in the prosencephalon including the future diencephalon (B). It was still expressed broadly in the early diencephalon (C at HH14 and D at HH18. C' and D' are the corresponding views of the same embryos from the dorsal side). The expression of *cNOT1* in the diencephalon at HH14 was confirmed by the OPT scan (H). At HH22, *cNOT1* expression became restricted to the visible protrusion of the pineal gland and a small region of the dorsal thalamic roof (E at HH22). *cNOT1* was expressed in the pineal gland and the posterior dorsal thalamic roof at HH25 (F, F' is the corresponding dorsal view of F, G is a sagittal section of F) A: anterior; P: posterior. The red arrow points to the region which is adjacent to pineal gland protrution and expresses *cNOT1*.

4.2.2 DKK1 is a pineal gland marker

In zebrafish, *cNOT1* homolog *flh* is a marker for pineal gland and is expressed in pineal precursors (Aquilina-Beck et al., 2007; Masai et al., 1997). However, from Figure 4.1, it shows that *cNOT1* was not only expressed in the pineal gland in chick embryo. Therefore, is there any pineal gland markers present in the chick embryo? In the developing diencephalon, expressions of *Wnt* family members were observed. For example, *Wnt4* is expressed broadly in the thalamus and epithalamus. But it was not expressed in the pineal gland. *Wnt8b* was expressed in the midline of the telencephalon and epithalamus. However, it is not expressed in the pineal body as well. (Quinlan et al., 2009) Therefore, it will be interesting to test the expression of Wnt antagonist in the pineal gland. One of the Wnt antagonists is Dickkopf-related protein1 (Dkk1) (Kawano and Kypta, 2003; Niehrs, 2006).

Expression of *DKK1* in chick embryos was analysed using whole-mount *in-situ* hybridisation at different stages of development. *DKK1* expression was observed from HH10 at the posterior procensephalon (future diencephalon) (Figure 4.2.A). At HH14, *DKK1* was expressed in a small region in the centre of the roof of the dorsal thalamus (Figure 4.2.B and B'). At later stages, *DKK1* expression was observed only within the pineal gland protrusion (Figure 4.2.C, D and D'). Therefore, *DKK1* is expressed only within the pineal gland, when the pineal body was morphologically observed.

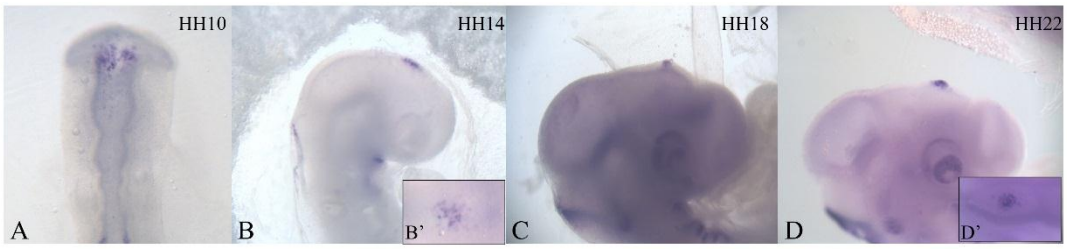


Figure 4.2 *DKK1* expression in the developing diencephalon studied by whole-mount *in-situ* hybridisation. *DKK1* started to be expressed in the centre of the future diencephalon from HH10 (A). This region becomes more compact at HH14 (B) and at HH18 (C). B' is the corresponding dorsal view of the embryo of B. *DKK1* was restricted to the pineal gland protrusion from HH22 (D; D' is the dorsal view of the embryo of D). All pictures were taken from the dorsal view of the embryo.

4.2.3 Fate mapping of the developing diencephalon

From the results in chapter 4.2 and 4.3, it was shown that *cNOT1* was not only expressed in the pineal gland but also in the posterior roof of the dorsal thalamus. In comparison, *DKK1* was expressed in the pineal gland specifically in chick embryo. To further confirm whether those *cNOT1*-expressing cells are pineal precursors or *cNOT1* expression is regulated during the development of the pineal gland, fate mapping of the roof of the dorsal thalamus was required. DiI staining was performed as described in chapter 2.5. To focus on the study of the development of the dorsal thalamus, fate mapping was performed at HH14, before the pineal gland can be morphologically observed. In addition, labelling the diencephalon at earlier stage (HH10-12) ended up with larger spread of DiI at later stage and it was more difficult to label the neuroepithelium (data in Appendix 3).

4.2.3.1 Standardisation of the coordinates of DiI labelled cells

Due to the 3D structure of brain vesicles, it was difficult to determine the coordinates of the DiI labelled cells. Figure 4.3 shows how the position of DiI labelled cells varies in different angles of viewing. The white line shows the distance of DiI labelled cells to the midline of the dorsal thalamus from ventral and lateral views, and from any random angle. This distance changes depending on the angle of observation. Therefore, to minimise this variation and measure the coordinates of the labelled cells as accurate as possible, the distances of the labelled cells to morphological landmarks in brain vesicles were measured. These included the telencephalon/diencephalon boundary, D2/D3 boundary (future thalamus and pretectum) (Figdor and Stern, 1993), mesencephalon/diencephalon boundary, and the margins of the brain vesicle. Figure 4.4 shows the landmarks seen from the dorsal view of the dorsal thalamus at HH14 and black lines show all of the distances to the morphological margins measured after DiI was labelled at HH14. These distances were measured again after incubation to HH22. Due to the difficulty of taking pictures of the *in vivo* embryo from the top view, images of the embryos before incubation were taken from lateral view only, to confirm the spread of the DiI label. Figure 4.5 shows the picture of DiI labelled embryo before incubation at HH14 (A-C) and after incubation to HH22 both from a lateral view (D-F) and from a dorsal view (G-H). These pictures show how the DiI labelled cells dispersed before and after incubation and helped to trace the cell fates. Using these methods in combination, coordinates of the DiI labelled cells were marked down for later fate mapping analysis.

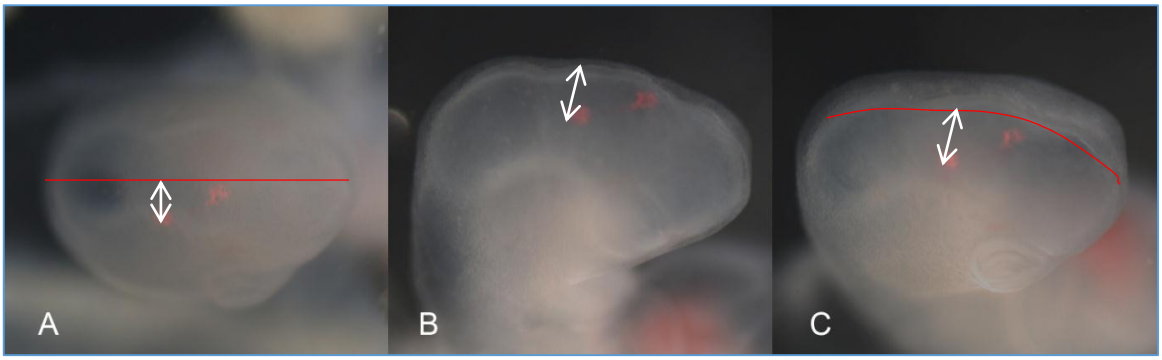


Figure 4.3 Coordinates of DiI labelled cells to the dorsal midline vary in different angles of viewings. An embryo at HH14 was labelled by DiI and images were taken from different angles. A) Dorsal view. B) Lateral view. C) Oblique view. White arrows indicate the distance between DiI labelled cells to the dorsal midline. Hence, the coordinates of DiI labelled cells vary based on the way of viewing and it is important to standardise the method of measurement.

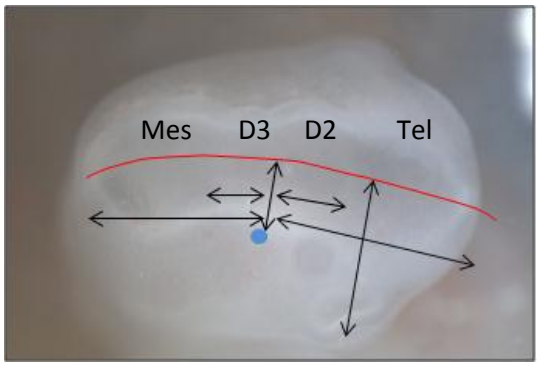


Figure 4.4 Standard method of measurement of DiI labelled cells at HH14 embryo. Distances between target cells (blue spot) and landmarks in the diencephalon were measured as shown in black lines. Land marks in HH14 brain included the dorsal midline (Red line), pretectum (D3)/mesencephalon (Mes), and thalamus (D2)/telencephalon (Tel). The anterior and posterior edges of the embryo were also measured as a reference.

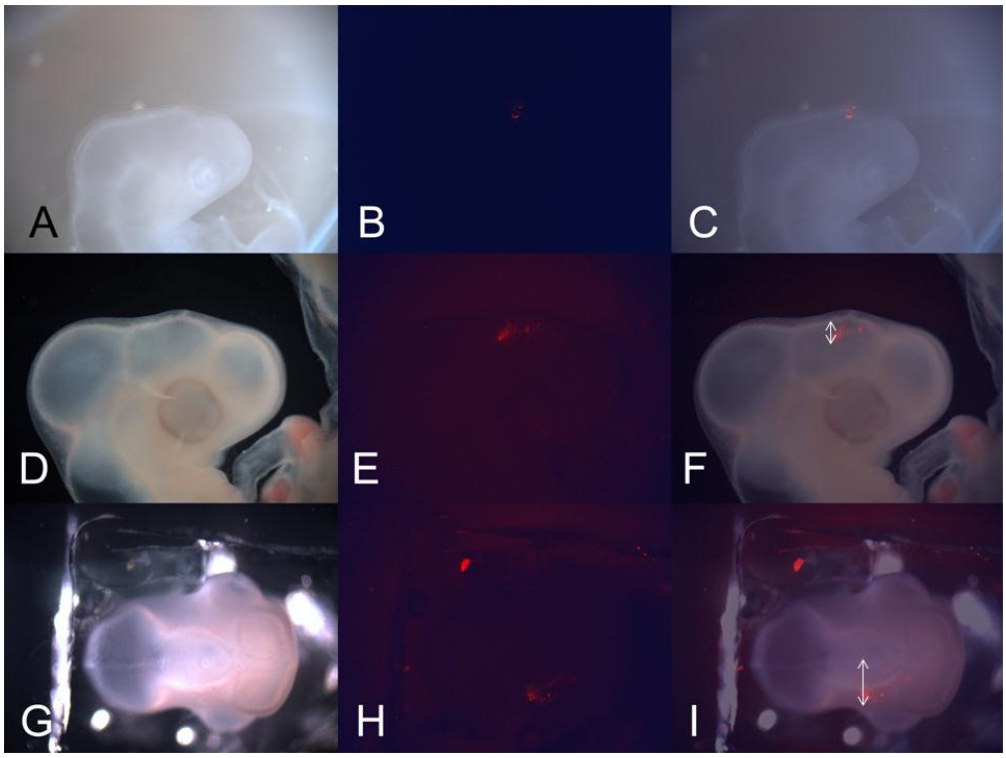


Figure 4.5 The dispersion of DiI label is analysed by microscope imaging. A-C: lateral views of the embryo taken immediately after labelling (HH14). D-F: lateral views of the embryo after incubation (HH22). G-I: dorsal views after incubation (HH22). All pictures are from the same embryo. A,D and G: incident epi-illumination. B, E and H: fluorescence (green excitation, red emission). C,F and I: merged bright field and fluorescence images. DiI labelled cells are traceable and the coordinates of labelled cells can be measured out. The successful DiI label should not disperse through the whole brain vesicle.

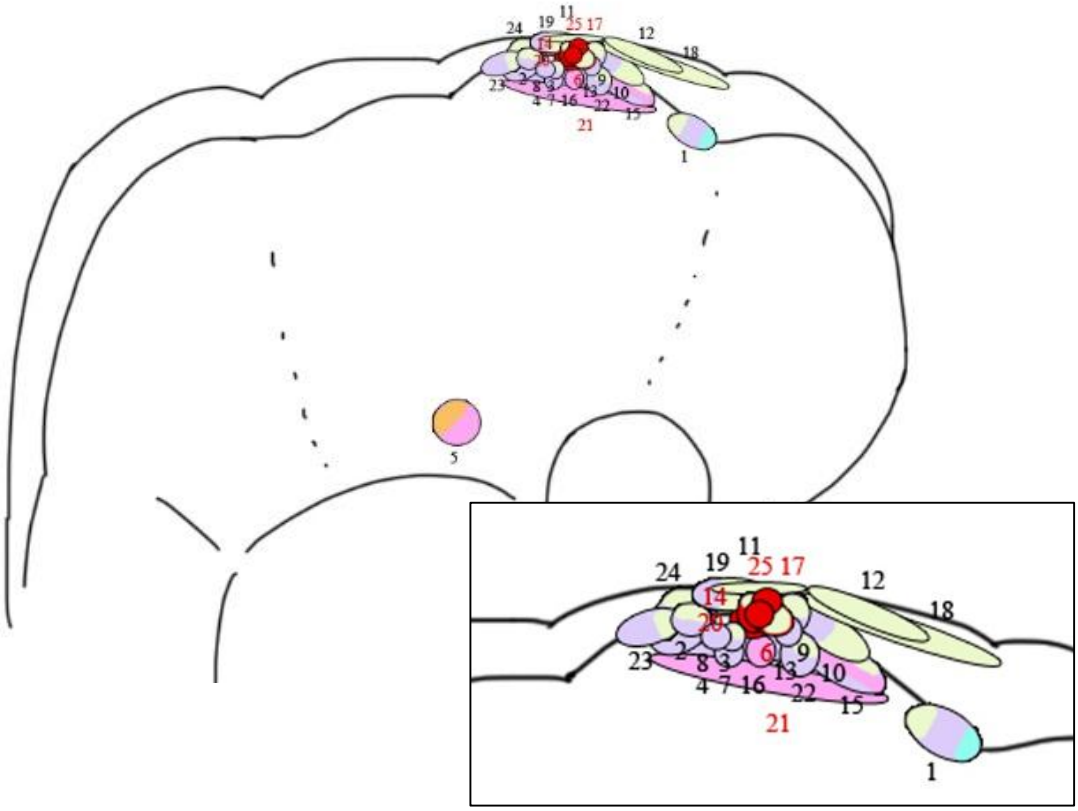
4.2.3.2 Correlation between cell fate and cNOT1 expression

248 embryos were labelled with DiI using the method described in Chapter 2.5. Those embryos that were 1) dead or showing abnormal structures after incubation, 2) DiI dispersed through the whole brain vesicle, 3) not at HH14 when labelled were discarded. A total of 74 embryos were retained for construction of the fate map. The raw data of these 74 embryos are included in appendix 2. For each embryo, the coordinates of DiI label before and after incubation were measured as described in Chapter 4.2.3.2. This data was combined with the picture taken to draw the fate map for each embryo. The final data is shown in Figure 4.6 (A-C). Each dot

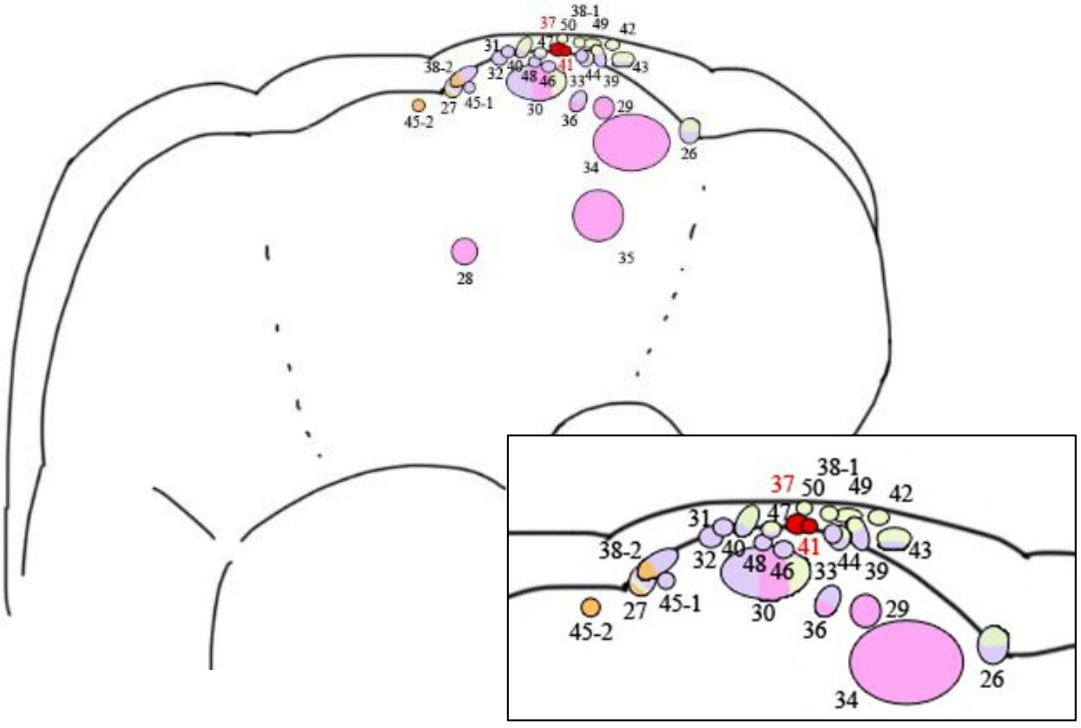
represents one label from one embryo. The drawings show the position of DiI label and the number of each label correlates to the raw picture in appendix. The colour represents the cell fate of each label, which correlates with the colour key showing in Figure 4.6 D. During the labelling process, the epidermis was easily labelled as well and causes a spread of colour after incubation at the surface of the embryo. As it was difficult to distinguish the label in the epidermis and the neuroepithelium under the microscope, if any epidermis was labelled in addition to the neuroepithelium, this DiI labelled epidermis was not excluded from the map. Also, although a boundary is drawn for the epithalamus in Figure 4.4 and 4.6.D, no morphological landmark exists for this region at this stage. The position of this line was based on the published expression of *CYP1B1*, which was suggested to be an epithalamus marker at HH15 (Guinazu et al., 2007) and *Fz2* that is expressed in the epithalamus at HH19 (Quinlan et al., 2009) .

Considering all of the limitations mentioned above and with careful labels, I can confirm that there was only a small proportion of cells located within the centre of the dorsal thalamus which contributed to the pineal gland tissues. It is shown as red spots in Figure 4.6 (A-C). And it was also clear that DiI labelled cells at the epithalamus surrounding the pineal (purple spots; also future habenula) were expressing *cNOT1*. Although the actual boundary of *cNOT1* expression within the epithalamus was difficult to define, due to: firstly, the strength of gene expression varied in each embryo and secondly, there was shrinkage of the embryo after the whole-mount *in-situ* hybridisation process.

A


24 19 11 25 17 12 18
14
20
23 2 8 3 6 9 13 10
4 7 16 22 15
21
1
5

B


38-1 37 50 49 42
31 47 41 43
38-2 32 40 48 46 33 44 39
45-2 27 45-1 30 36 29
34 26
35
28

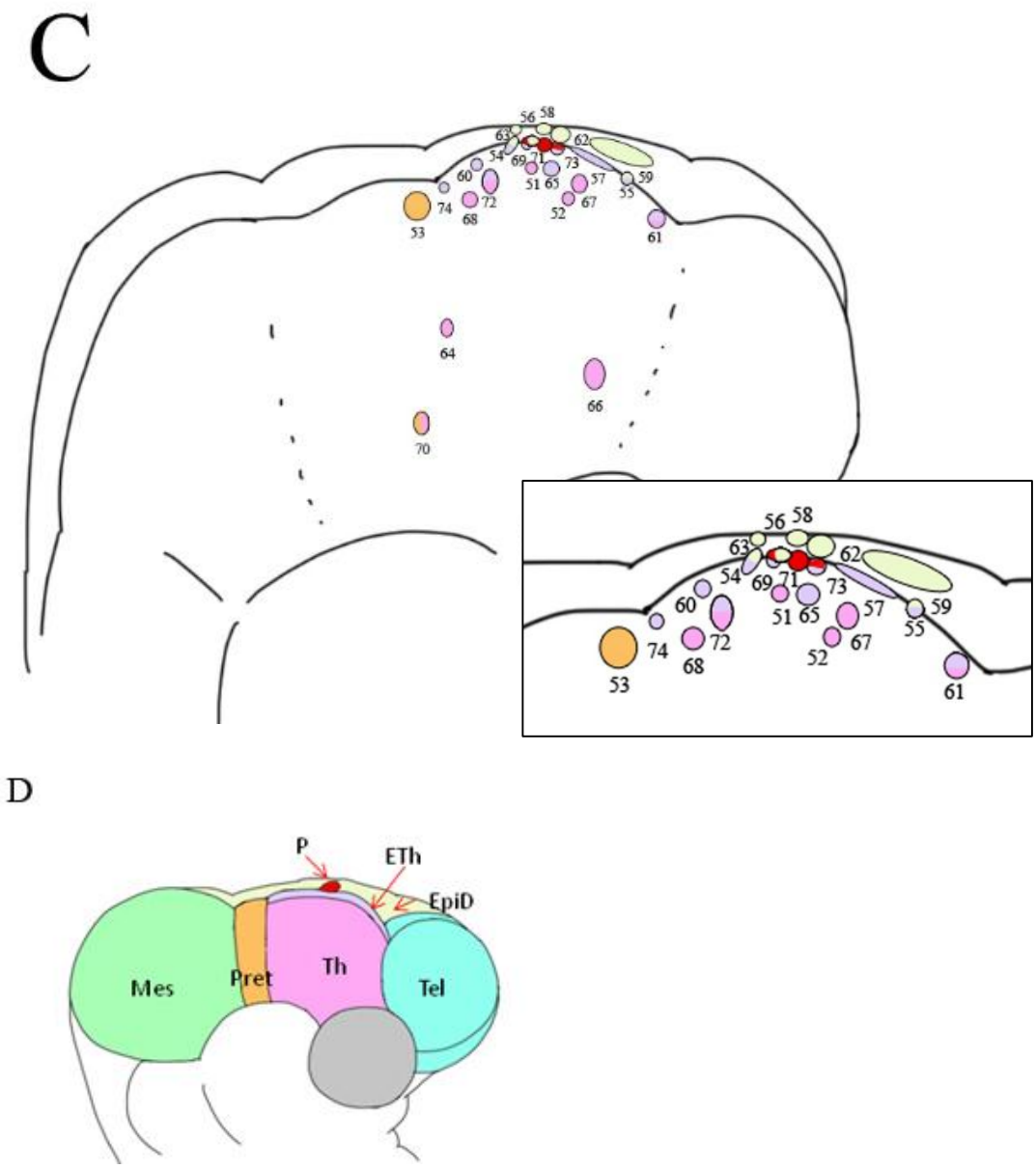


Figure 4.6 Fate mapping of diencephalic cells. The fate maps constructed from all 74 embryos at HH14 are summarised in pictures A to C (25 labels in A and B, and 24 labels in C). Each DiI dot represents the position of a labelled group of cells, and the colour of the dot symbolises the structures (D, for a stage 22 brain) to which the labelled cells contributed when analysed at stage 22. The small pictures next to each diagram show a corresponding enlargement of the dorsal thalamus, which was the *cNOT1* expression region at HH14. The multiple colours in one spot means cells in this area contributed to both regions. All heads are drawn in the same direction with posterior on the left and anterior on the right hand side. Eth:epithalamus; Tel:telencephalon; Mes:mesencephalon; Pret:pretectum; Th:thalamus; EpiD:epidermis; P:pineal gland.

4.3 Discussion

At HH14, the roof of the dorsal thalamus is not differentiated yet. Although *CYP1B1* is considered to be an epithalamus marker at HH15 (Chambers et al., 2007; Guinazu et al., 2007), there are no morphological boundaries between the roof and the lateral side of the dorsal thalamus at this stage. As the epithalamus develops, it subdivided into the habenula and the pineal gland which are conserved structures in vertebrates. It is known to be involved in the central nervous system. It receives signals from the cortex, limbic system and basal ganglia through the stria medullaris and sends signals out to the midbrain and hindbrain through the fasciculus retroflexus (Hikosaka et al., 2008; Sutherland, 1982). In many lower vertebrates, the habenula exhibits a left-right asymmetry and markers of genes are identified both for symmetric and asymmetric structures. For example, in zebrafish, *cpd2* is a symmetric marker and *lov* is an asymmetric marker for the habenula (Gamse et al., 2003). However, no specific marker is found for the habenula in higher vertebrate yet. The gene expressions in the epithalamus are mainly shared with the pretectum and thalamus (Quinlan et al., 2009). To compare, genes have been identified in the development of habenula neuronal traits and *Pou4f1* is one of the sample which marks postmitotic habenular neurons (Quina et al., 2009).

As little is known about the development of the epithalamus and there is a lack of a specific marker for the epithalamus in chick embryo, it is important to understand the specification of epithalamus structures both in terms of gene expressions and cell fate before study of the functions in development. *cNOT1* was shown to be expressed broadly in the early diencephalon (Figure 4.1), which raises a possibility that it may be a marker for the pineal precursor. In zebrafish, *cNOT1* homolog *flh* is shown to express specifically in the pineal gland and is involved in the regulation of the development of the pineal (Snelson et al., 2008; Talbot et al., 1995). However, whole-mount *in-situ* analysis of *cNOT1* shows that it was not only expressed in the pineal gland but also the posterior epithalamus to the pineal (Figure 4.1). Also, the fate map of the thalamus shows that only a small proportion of *cNOT1* was contributed to the pineal gland (Figure 4.7). Therefore, *cNOT1* expression is down-regulated during the development of thalamus. On the other hand, *DKK1*, which is

a Wnt antagonist, was shown to be a possible pineal gland marker. Whole-mount *in-situ* hybridisation of *DKK1* shows that it was expressed only in the pineal protrusion (Figure 4.2). However, as the *DKK1* expressing region was very small at the centre of dorsal thalamus at HH14, it was difficult to identify whether they are all pineal gland progenitor by comparing to the fate map in Figure 4.6.

So far, fate maps for chick diencephalon have been done at HH8 (Couly and Le Douarin, 1985; Couly and Le Douarin, 1987) and HH10 (Garcia-Lopez et al., 2004) respectively by quail-chick transplantation. These studies reveal the progenitors for compartments in the diencephalon, including the epithalamus, thalamus and pretectum. However, the progenitors for both the pineal gland and habenular are not defined in chick embryo yet. Fate maps have also been done for mouse and *Xenopus* to identify precursors for each brain vesicles, but pineal gland and habenular origins are not defined yet (Eagleson and Harris, 1990; Inoue et al., 2000). In comparison, fate mapping in zebrafish have revealed the pineal precursor which expresses *flh* specifically (Kimmel et al., 1990; Kozlowski et al., 1997; Woo et al., 1995). Therefore, the fate map of HH14 diencephalon in this chapter provides information about pineal gland precursors and the development of the epithalamus in chick embryos.

Chapter 5

Pineal Gland is Regenerated after Ablation of Progenitor Cells

5 Pineal Gland is Regenerated after Ablation of Progenitor Cells

5.1 Introduction

To test whether the pineal region is an organizer, one of the methods is to remove the potential organizer and study the changes of surrounding tissues both in terms of morphology and gene expressions. In the mouse embryo, ablation of the organizer node at late gastrulation causes a deformation of somites, absence of notochord and floor plate and foreshorten of the body axis, which demonstrates that the mouse node is crucial for patterning of the ventral neural tube and lateral body axis (Davidson et al., 1999). The node-ablated embryo also expresses *Pitx2* symmetrically, which is a left-right asymmetry marker (Davidson et al., 1999). However, the embryonic stage when ablation is performed is important as regeneration may occur. For example, the node is regenerated when it is ablated between HH3+ and HH4- in chick embryo (Grabowski, 1956; Psychoyos and Stern, 1996). The regenerated node is identical to that in normal embryos and the embryo develops normally after ablation. Truncated notochord is observed when the node is ablated at HH4+ or later (Grabowski, 1956; Joubin and Stern, 1999; Psychoyos and Stern, 1996; Yuan et al., 1995). Hence, before studying whether the potential organizer can influence the patterning of surrounding tissues, the following questions have to be answered: 1) whether all organizer cells have been removed; 2) which embryonic stage is most appropriate to perform ablation experiments; 3) whether organizer is regenerated after ablation.

Based on the gene expression analysis in Chapter 3, the roof of the dorsal thalamus is a potential organizer with the expression of syn-expressed genes *cNOT1*,*TSPAN6,* and *PKIγ*. The expression of *cNOT1* starts in a broad area and later on restricts to the pineal gland, which raised the possibility that *cNOT1*-expressing cells may be the pineal gland progenitors. However, fate mapping studies in Chapter 4 shows that not all *cNOT1* cells develop to the pineal gland. Therefore, there is a question: whether the pineal gland or the *cNOT1*-expressing territory is a potential organizer. To address this question, ablation experiments were performed for these two regions respectively.

This Chapter presents the results of ablation of the pineal region and how they are related to the organizing activities and the regeneration potential of this region.

5.2 Results

5.2.1 Pineal gland is regenerated after ablation of pineal progenitors.

Ablation experiments were performed as described in Chapter 2.6. Ablated embryos were processed to whole-mount *in-situ* hybridisation as described in Chapter 2.2.6.

To test the roles of pineal gland and the *cNOT1*-expressing cells in brain development, ablation of the roof of the diencephalon at HH14 was performed. The roof of the diencephalon was divided into three parts as it is shown in Figure 5.1. "Anterior" (A) mainly includes cells that are located in the anterior of the roof of the dorsal thalamus. "Centre" (C) is the centre region of the roof of the dorsal thalamus. "Posterior" (P) is the group of cells locating at the posterior part of the roof of the dorsal thalamus. Based on the data from whole-mount *in-situ* in Chapter 3 and the fate map in Chapter 4, the anterior cells contain those that express *cNOT*1 and not pineal progenitors. The centre region of the roof of the dorsal thalamus expresses *cNOT1* and contains pineal progenitors as well. On the other hand, the posterior tissues of the roof of the diencephalon express *cNOT1* but do not contribute to the pineal gland.

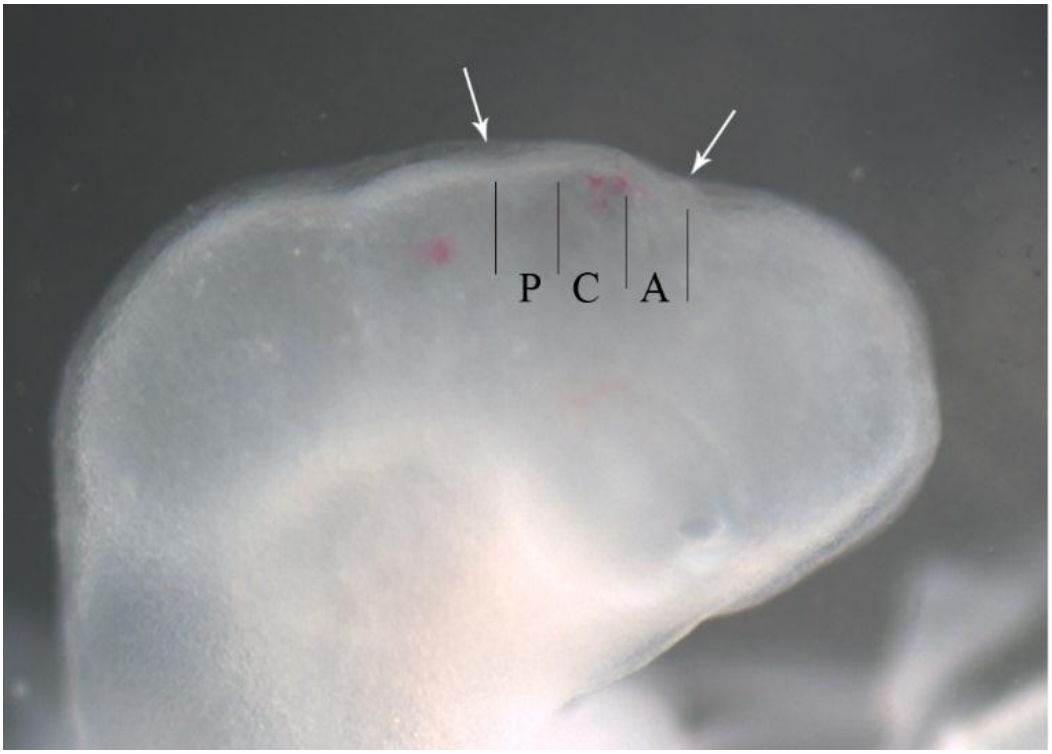


Figure 5.1 Design of ablation experiment. The picture shows the landmark of a HH14 embryo used for ablation experiments. The division is based on the measurement using graticules. P stands for the posterior tissues in the roof of dorsal thalamus. C is the centre of the roof of dorsal thalamus. A is anterior tissues in the roof of dorsal thalamus. The white arrows point to the morphological land mark between telencephalon and thalamus, and between thalamus and pretectum.

Based on the fate map and gene expression analysis in Chapter 4, it showed that pineal gland progenitors contain a small group of cells located in the centre of the roof of the dorsal thalamus at HH14 ("center" region in Figure 5.1). *cNOT1* was expressed in a large region at HH14 in the diencephalon including the pineal progenitors ("center" and "posterior" region in Figure 5.1). To examine whether the pineal region is required for patterning surrounding tissue, the "center" region containing pineal progenitors was removed at HH14 embryos and the ablated embryos were incubated for two days. Ablation of pineal progenitors (center) was also combined with removal of either posterior or anterior tissue, to see whether *cNOT1*-expressing cells are required for development. Before processing embryos for further analysis, regeneration of pineal gland was observed. As it is shown in

Table 5.1, removal of the “centre” region containing pineal progenitors alone led to regeneration of the pineal gland protrusion in all cases. Removal of pineal progenitors with the neighbouring anterior region led to regeneration of the pineal gland protrusion in half of the cases. On the other hand, removal of pineal progenitors together with the posterior neighbouring tissue gives no regeneration of the pineal gland protrusion in all cases. As the “centre” and “posterior” parts of the roof of the dorsal thalamus contain nearly all the *cNOT1* expressing cells, it raised the possibility that the pineal gland can regenerate from *cNOT1* expressing cells that are not pineal progenitors.

However, this preliminary work was quick ablation without picturing and was only based on the observation of the morphological protrusion of pineal gland. It was not precise enough and do not show whether the gene expression in the roof of diencephalon was still the same after ablation. Therefore, further analysis was carried out and will be discussed in the next chapter.

Regions of Ablation	number of Regeneration
Centre (C)	5/5
Center+Anterior (C+A)	3/6
Center+Posterior (C+P)	0/6

Table 5.1 preliminary data of ablation experiments in dorsal thalamus. The first row shows the number of regeneration, when the centre of the roof of dorsal thalamus was ablated. Five out of five samples got regeneration. The second row shows the number of regeneration, when the centre and the anterior tissues of the roof of dorsal thalamus was ablated. Half of the samples had pineal regeneration. The third row shows the number of regenerations, when the centre and the posterior of the roof of dorsal thalamus was ablated. None of the samples had pineal regeneration.

5.2.2 Gene expression patterns are normal after regeneration of pineal

Apart from the morphology of the diencephalon, the gene expression patterns needed to be examined after regeneration of the pineal gland. As it shows in Figure 5.2, removal of pineal progenitors (region "C") alone at HH14 led to the regeneration of the pineal gland protrusion by 48hours incubation, which correlates to Table 5.1. Whole-mount *in-situ* hybridisation was performed to further test the expression of pineal region markers after ablation. *cNOT1* was expressed both in the pineal gland protrusion and in the posterior tissues, as in their usual pattern, 48 hours post-surgery (Figure 5.2c). *Wnt8b* and *Pax6* are genes expressed in diencephalon and telencephalon from about HH14 (Figure 5.2 d,e,g & h). Ablation of the pineal gland at HH14 removed part of the *Wnt8b* and *Pax6* expressing tissues, however their expressions were still detected around pineal two days after surgery (Figure 5.2 f & i). The expression area of *Pax6* was slightly smaller in ablated embryo, which may be due to the unfinished regeneration process. Therefore, the pineal region (both *cNOT1* expressing cells and the pineal gland protrusion) can regenerate after removal of pineal progenitors in the "centre" of the roof of dorsal thalamus at HH14 and as a consequence, patterning of the diencephalon occurs normally.

5.2.3 Ablation of all cNOT1 expressing cells prevents the regeneration of the pineal gland and DKK1 expression

To examine whether the pineal gland regenerates from surrounding *cNOT1* -expressing cells, ablation experiments were performed to remove the entire cNOT1 expressing region. To see whether *cNOT1*-expressing cells were removed completely, embryos from which "Centre" and "Posterior" tissues had been ablated were fixed immediately after ablation. No *cNOT1*-expressing cells were observed (Figure 5.3b). The ablated embryos were also tested for the expression of *DKK1*, which is expressed only in the pineal gland (Chapter 4). No *DKK1* expression was observed (Figure 5.3d). Another group of ablated embryos was incubated for two days to see whether the pineal gland would be regenerated. As shown in Figure

5.3h, no *DKK1* expression was observed. Also, there was no pineal gland protrusion. Therefore, the pineal gland did not regenerate. The expression of *cNOT1* was also tested, and it showed that *cNOT1* was re-expressed in a small region of the roof of diencephalon (Figure 5.3f). However, no morphological protrusion was observed. Therefore, removal of all *cNOT1* expressing cells in the "Centre" and "Posterior" tissues of the diencephalon prevents regeneration of the pineal gland protrusion that normally expresses *DKK1*. However, *cNOT1* expression itself regenerates after ablation.

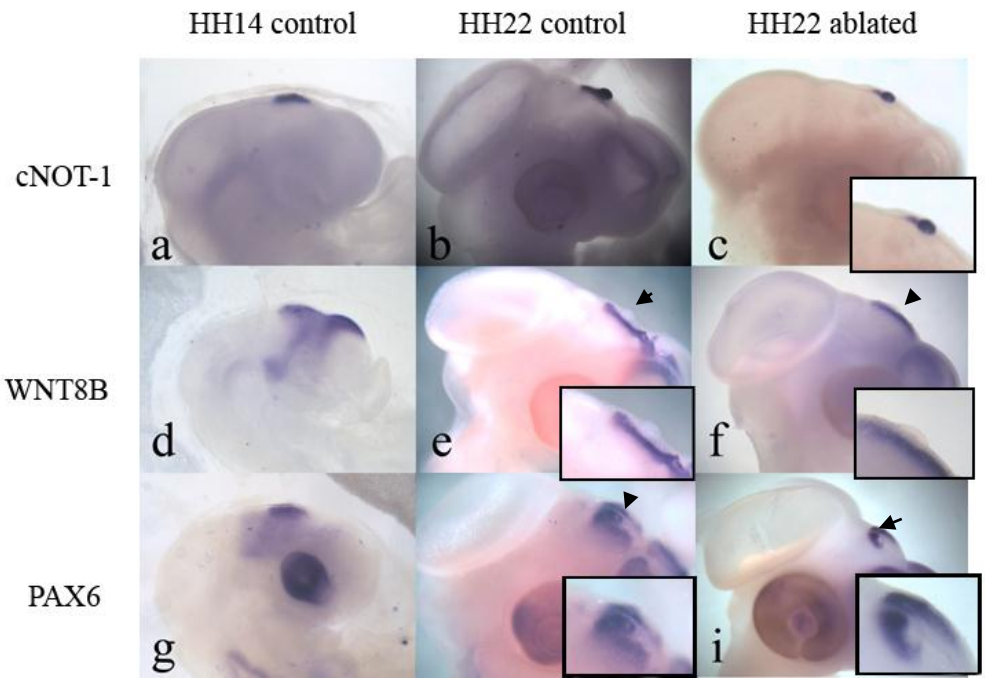


Figure 5.2 Ablation of pineal progenitors ("centre" region) was performed at HH14 and embryos were incubated two days (~H22) before process to whole-mount *in-situ* hybridisation. The first row shows the expression of *cNOT1* in normal HH14 embryo (a), normal HH22 embryo (b) and HH22 ablated embryo (c). Smaller pineal protrusion was observed in (c) with *cNOT1* expression, which means that pineal was regenerated. The second row shows the expression of *Wnt8b* in normal HH14 embryo (d), normal HH22 embryo (e), and HH22 ablated embryo (f). *Wnt8b* was not expressed in pineal gland at HH22 but in surrounding tissues. There was no difference in *Wnt8b* expression between the control embryo and ablated embryo. The third row shows the expression of *Pax6* normal HH14 embryo (g), normal HH22 embryo (h) and HH22 ablated embryo (i). *Pax6* was not expressed in pineal gland at HH22 but in surrounding tissues. The ablated embryo had a smaller area of *Pax6* expressed around the pineal, which may due to incomplete regeneration. Black arrows are pointing at the transparent pineal.

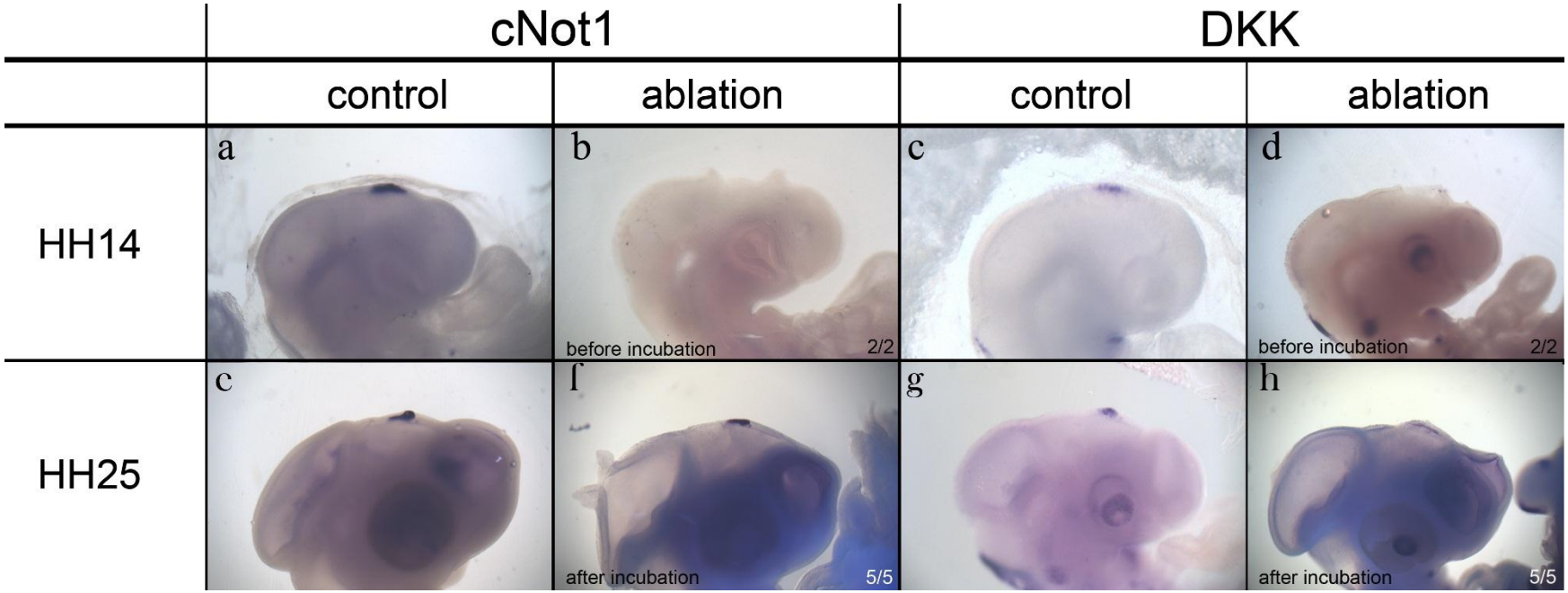


Figure 5.3 Ablation of *cNOT1* expressing cells ("centre" and "posterior" regions). Pictures a, c, e and g are controls for *cNOT1* and *DKK1* at either HH14 or HH25 respectively. Embryos were ablated with centre and posterior tissues of the roof of dorsal thalamus based on Figure 5.1 scale. Ablated embryos were either fixed directly to check gene expression (b & d) or processed to incubation for regeneration (f & h). It shows that the ablation removed all *cNOT1* and *DKK1* expressing cells (b & d). The number accounts for the embryos that had no *cNOT1* or *DKK1* cells left, i.e. successful ablation. The ablated embryos were harvested two days later and were processed to whole-mount *in-situ*. None of the embryos had pineal gland regeneration. *DKK1* expression was not detected in all samples (h, number stands for no *DKK1* expression, i.e. successful ablation). In comparison, as shown in picture f, *cNOT1* was expressed again without regeneration of the pineal (no protrusions). The number stands for the embryos that had *cNOT1* expression but no regeneration. Therefore, re-expression of cNOT1 was a consistent result.

5.4 Discussion

As discussed in Chapter 3, the roof of the dorsal thalamus with *cNOT1* expression was revealed as a possible organizer region. Fate mapping experiments (Chapter 4), showed that this region contains both pineal progenitors and cells with non-pineal, epithalamic fate, i.e. *cNOT1* is not only expressed in pineal progenitors. This region expresses *cNOT1*, *PKIγ* and *TSPAN6* and lacked expression of some of the genes generally absent from the tested organizers (*MSX1*&*2*, *DLX5*&*6*,*BTG2*, etc.) at HH14 (Chapter 3) and was therefore a possible organizer. To test for organizing properties of this region, various territories of the roof of the dorsal thalamus were ablated. First, an attempt was made to remove just the pineal progenitors, located at the centre of the roof of the dorsal thalamus. However, ablation of pineal precursors (centre, with *DKK1* expression) showed regeneration of the pineal gland with normal morphology and diencephalic gene expression patterns (Table 5.1 and Figure 5.2). Therefore, the pineal gland can be regenerated from surrounding tissues. To confirm this, different regions from the surrounding epithalamic area were removed along with pineal progenitors. Removal of the central and anterior regions still led to regeneration of the pineal gland protrusion (Table 5.1). The ablation removed all pineal progenitors but left tissues (posterior) that expressed *cNOT*1. In contrast, removal of all pineal progenitors together with posterior tissues containing virtually all *cNOT1* expressing cells reduced the incidence of morphological regeneration (Table 5.1and Figure 5.3). Although the pineal marker *DKK1* was not expressed and a pineal gland protrusion was not observed after ablation of the centre and posterior regions of the dorsal diencephalon , expression of *cNOT1* was restored (Figure 5.3). Therefore, the pineal gland can be regenerated from surrounding tissues that have *cNOT1* expression and it raises a question that whether *cNOT1* plays a role in development and regeneration of the pineal gland. In zebrafish, *flh* (which is homologue to NOT) mutation can affect pineal development without disruption of the neighbouring parapineal (Snelson et al., 2008), suggesting that *Not* function is important for pineal development. However, there are another *NOT* gene in chick, which is *cNOT2*. Comparing to *cNOT1*, *cNOT2* is more colsely related to zebrafish *flh (Stein et al., 1996)*. Therefore, studies of both *cNOT1* and *cNOT2* are required to address the role of *NOT* gene in pineal regeneration. Further more, pienal gland is not differentiated at HH14, i.e. neuroepithelial cells are

identical within the dorsal diencephalon (Calvo and Boya, 1978; Calvo and Boya, 1979). Ablation of differentiated pineal cells is required to examine whether *cNOT1*-expressing cells can regenerate the pineal gland at later stages. Ablations of the pineal area at later stages (>HH18) were tried. However these embryos did not survive, probably due to haemorrhage caused by damage to the surrounding blood vessels that have developed by this stage. Embryos without a pineal gland appeared to develop normally to HH22 and have apparently normal morphology of the head, but a more detailed study of axon tracts and gene expression is required to ascertain this rigorously. Finally, there is a question of whether the posterior non-pienal progenitor, *cNOT1*-expressing cells are required for normal developmen of the pineal. Ablation of the posterior dorsal thalamus is required to address this question. Therefore further experiments are needed to be done in this case.

In conclusion, the ablation experiements showed that the pineal gland can regenerate after ablation at stage 14 and that patterning of the diencephalon occurs normally, provided that posterior *cNOT1*-expressing cells are spared at the time of ablation. Removal of all *NOT1*-expressing cells prevents regeneration of the pineal but c*NOT1* expression returns. Therefore, to test the organizing ability of the c*NOT1*-expressing region of the roof of the dorsal thalamus, further experiments are required. The following questions can be studied in the future.

1) Whether regeneration will appear if ablation of the pineal gland is performed at later stages, i.e. after HH18 when the pineal gland is more differentiated?
2) Can transplantation of the pineal gland or *cNOT1*-expressing cells to ectopic site in brain region cause misexpression of brain markers, like *Wnt8b*, *Wnt4*, *Pax6* and *Otx2*?
3) Whether gain- or loss-of-function of *cNOT1* affects the development of the pineal gland and/or diencephalon?

Chapter 6

General Discussion

6 General Discussion

The expression of genes that are enriched or depleted in 3 known organizers (Chapter 3) pointed to the region of the roof of the dorsal thalamus including and adjacent to the pineal gland as a potential organizer. This region expresses 3/32 of the enriched genes and lacks expression of 12/16 depleted genes which, although not representing the majority of the genes within this synexpression group, includes a key transcription factor, *cNOT1*, which is only expressed in a few regions of the embryo, most of which are known to have signalling activity: the node at stage 3+-4, the notochord from stage 5 onwards and the posterior limb bud. This raised the possibility that the peri-pineal region of the thalamic roof could be an organizer of surrounding regions of the brain.

To explore this further, we started by constructing a fate map of this regions (Chapter 4) at stage 14. *cNOT1* is expressed from stage 8 at the prospective diencephalon, at stage 10 as a broad region in the diencephalic roof, and expression gradually becomes confined to the emerging pineal protrusion (visible from stage 18) and a territory just posterior to it on the dorsal thalamic roof. DiI-labelling reveals that not all cells within the *cNOT1*-expressing domain are pineal gland progenitors. In comparison, *flh* which is *cNOT* homologue, define pineal gland progenitors, which is confirmed by mutation of *flh* that cause the pineal complex to premature at 18 somite stage (Masai et al., 1997). The expression pattern of *cNOT1* and the contribution of *cNOT1*-expressing cells to the diencephalon I found corresponds more closely to the observation from Staudt and Houart in zebrafish, where *cNOT1* is expressed in a broad area in the epithalamus and telencephalon and only a small proportion of *cNOT1*-expressing cells participate in the pineal gland (Staudt and Houart, 2007). However, *DKK1* is expressed exclusively in the pineal gland from stage 14 in the chick embryo, prior to morphological changes that reveal the appearance of the pineal gland (Calvo and Boya, 1978; Calvo and Boya, 1979). Comparing the expression patterns and fate maps, the majority of *DKK1*-expressing cells develop into the pineal gland, while only a small proportion of *cNOT1*-

expressing cells contribute to the pineal gland and most of them develop into the surrounding epithelium of thalamus.

To determine whether it is the pineal gland or the *cNOT1*-expressing territory that acts as an organizer, one approach is to establish whether it is required for patterning the surrounding diencephalon. However, ablation could lead to regeneration, which would undermine this experiment. The broad expression of *cNOT1* in the early diencephalon and the findings (Chapter 4) that not all *cNOT1*-expressing cells contribute to the pineal also raises the question of whether non-pineal precursor *cNOT1*-expressing cells can restore the pineal gland when this is ablated. We performed ablations of the prospective pineal gland territory at stage 14 either alone or with the neighbouring anterior or posterior diencephalon roof (the latter contains most of the non-pineal *cNOT1*-expressing cells). It was found that regeneration of pineal gland was observed when only pineal precursors were removed, and was inhibited when both the precursors and the posterior diencephalon roof were removed. Therefore, the pineal gland can regenerate from surrounding diencephalic roof, provided that cells from the *cNOT1*-expressing territory are spared from ablation. Also, to further investigate the organizing ability of the pineal gland, regeneration can be inhibited by either removal of surrounding tissues or ablation at later embryonic stage. On the other hand, to test whether *cNOT1*-expressing territory is an organizer, ablation may be performed at later stage, though *cNOT1* is expressed broadly in the early diencephalon and removal of all *cNOT1*-expressing cells would require ablation of almost whole diencephalon roof.

Based on our ablation experiment, it is still clear that regeneration of the pineal gland requires *cNOT1* expression, as removal of all *cNOT1*-expressing cells prohibits the pineal gland regeneration. It is also interesting that *cNOT1* expression re-appeared without the regeneration of pineal, which means it is induced by the surrounding tissues. Comparing to the pineal, removal of the progenitors alone did not interrupt the normal development of the dorsal diencephalon and the *cNOT*-expressing region seems to be more important to the development. Therefore, if

there is an organizer within diencephalon, it is more likely to be the *cNOT*-expressing region. To further examine this possibility, transplantation of *cNOT*-expressing region to other brain vesicles, for instance, telencephalon or mesencephalon, is needed. If it is a true organizer, the transplantation of *cNOT*-expressing tissues to ectopic side should induce the expression of diencephalic markers. And this transplantation should be compared to that of pineal gland transplantation alone, to confirm the requirement of *cNOT1* expression for brain development. Also, the ablation experiments in Chapter 5 lacked the removal of posterior part of the dorsal thalamus alone. This posterior tissues contains *cNOT*-expressing cells but no pineal gland progenitors. If the *cNOT1* expression is needed for normal development, reduction of the expression region may affect the normal development of the pineal gland and diencephalon as well, which in turn, would prove the induction activity of *cNOT1*-expressing cells rather than regeneration.

In conclusion, the experiments reported in this thesis pointed to a region where gene expression patterns partly resemble those of three known organizers: the pineal and part of the adjacent thalamic roof. Ablation of the pineal precursors leads to regeneration as long as peri-pineal *cNOT1* cells are spared. To determine whether the pineal or the entire *cNOT1*-expressing region acts as an organizer it will be necessary to complete the ablation experiments, as well as transplantation experiments to more lateral, anterior and posterior sites, analysing markers for features in the neighbouring diencephalon (eg. *Wnt8b*, *Pax6, Lfng*, the pattern of axon tracts). If the pineal or peri-pineal region acts as an organizer, ablation should lead to loss of some aspects of the pattern and transplantation should lead to some features being duplicated. It will be important to perform these experiments at different stages.

Reference

Aardal, S. and Helle, K.B., 1992. The vasoinhibitory activity of bovine chromogranin A fragment (vasostatin) and its independence of extracellular calcium in isolated segments of human blood vessels. Regul Pept. 41, 9-18.

Abdelkhalek, H.B., Beckers, A., Schuster-Gossler, K., Pavlova, M.N., Burkhardt, H., Lickert, H., Rossant, J., Reinhardt, R., Schalkwyk, L.C., Muller, I., Herrmann, B.G., Ceolin, M., Rivera-Pomar, R. and Gossler, A., 2004. The mouse homeobox gene Not is required for caudal notochord development and affected by the truncate mutation. Genes Dev. 18, 1725-36.

Abercrombie, M. and Bellairs, R., 1954. The Effects in Chick Blastoderms of Replacing the Primitive Node by a Graft of Posterior Primitive Streak. J Embryol Exp Morphol. 2, 55-&.

Adams, K.A., Maida, J.M., Golden, J.A. and Riddle, R.D., 2000. The transcription factor Lmx1b maintains Wnt1 expression within the isthmic organizer. Development. 127, 1857-1867.

Al-Jassar, C., Knowles, T., Jeeves, M., Kami, K., Behr, E., Bikker, H., Overduin, M. and Chidgey, M., 2011. The nonlinear structure of the desmoplakin plakin domain and the effects of cardiomyopathy-linked mutations. J Mol Biol. 411, 1049-61.

Al Tassan, N., Khalil, D., Shinwari, J., Al Sharif, L., Bavi, P., Abduljaleel, Z., Abu Dhaim, N., Magrashi, A., Bobis, S., Ahmed, H., Alahmed, S. and Bohlega, S., 2012. A missense mutation in PIK3R5 gene in a family with ataxia and oculomotor apraxia. Hum Mutat. 33, 351-4.

Alappat, S., Zhang, Z.Y. and Chen, Y.P., 2003. Msx homeobox gene family and craniofacial development. Cell Res. 13, 429-42.

Alexandre, C., Jacinto, A. and Ingham, P.W., 1996. Transcriptional activation of hedgehog target genes in Drosophila is mediated directly by the cubitus interruptus protein, a member of the GLI family of zinc finger DNA-binding proteins. Genes Dev. 10, 2003-13.

Altaba, A.R., Placzek, M., Baldassare, M., Dodd, J. and Jessell, T.M., 1995. Early Stages of Notochord and Floor Plate Development in the Chick-Embryo Defined by Normal and Induced Expression of Hnf-3-Beta. Dev Biol. 170, 299-313.

Alvarado-Mallart, R.M., 1993. Fate and potentialities of the avian mesencephalic/metencephalic neuroepithelium. J Neurobiol. 24, 1341-55.

Amireault, P. and Dube, F., 2000. Cloning, sequencing, and expression analysis of mouse glucosamine-6-phosphate deaminase (GNPDA/oscillin). Mol Reprod Dev. 56, 424-35.

Andersson, L., Westerlund, J., Liang, S., Carlsson, T., Amendola, E., Fagman, H. and Nilsson, M., 2011. Role of EphA4 receptor signaling in thyroid development: regulation of folliculogenesis and propagation of the C-cell lineage. Endocrinology. 152, 1154-64.

Andres, A.C. and Ziemiecki, A., 2003. Eph and ephrin signaling in mammary gland morphogenesis and cancer. J Mammary Gland Biol Neoplasia. 8, 475-85.

Apostolova, G., Dorn, R., Ka, S., Hallbook, F., Lundeberg, J., Liser, K., Hakim, V., Brodski, C., Michaelidis, T.M. and Dechant, G., 2007. Neurotransmitter phenotype-specific expression changes in developing sympathetic neurons. Mol Cell Neurosci. 35, 397-408.

Appleton, B.A., Wu, P., Maloney, J., Yin, J., Liang, W.C., Stawicki, S., Mortara, K., Bowman, K.K., Elliott, J.M., Desmarais, W., Bazan, J.F., Bagri, A., Tessier-Lavigne, M., Koch, A.W., Wu, Y., Watts, R.J. and Wiesmann, C., 2007. Structural studies of neuropilin/antibody complexes provide insights into semaphorin and VEGF binding. EMBO J. 26, 4902-12.

Aquilina-Beck, A., Ilagan, K., Liu, Q. and Liang, J.O., 2007. Nodal signaling is required for closure of the anterior neural tube in zebrafish. BMC Dev Biol. 7, 126.

Araki, M., Nagata, K., Satoh, Y., Kadota, Y., Hisha, H., Adachi, Y. and Taketani, S., 2002. Developmentally regulated expression of Neuro-p24 and its possible function in neurite extension. Neurosci Res. 44, 379-89.

Artinger, K.B. and Bronnerfraser, M., 1993. Delayed Formation of the Floor Plate after Ablation of the Avian Notochord. Neuron. 11, 1147-1161.

Babu, D. and Roy, S., 2013. Left-right asymmetry: cilia stir up new surprises in the node. Open Biol. 3, 130052.

Bach, I., 2000. The LIM domain: regulation by association. Mech Dev. 91, 5-17.

Bach, I., Carriere, C., Ostendorff, H.P., Andersen, B. and Rosenfeld, M.G., 1997. A family of LIM domain-associated cofactors confer transcriptional synergism between LIM and Otx homeodomain proteins. Genes Dev. 11, 1370-80.

Bachiller, D., Klingensmith, J., Kemp, C., Belo, J.A., Anderson, R.M., May, S.R., McMahon, J.A., McMahon, A.P., Harland, R.M., Rossant, J. and De Robertis, E.M., 2000. The organizer factors Chordin and Noggin are required for mouse forebrain development. Nature. 403, 658-661.

Bachvarova, R.F., Skromne, I. and Stern, C.D., 1998. Induction of primitive streak and Hensen's node by the posterior marginal zone in the early chick embryo. Development. 125, 3521-34.

Baker, R.K. and Antin, P.B., 2003. Ephs and ephrins during early stages of chick embryogenesis. Dev Dyn. 228, 128-42.

Baldock, R.A., Bard, J.B., Burger, A., Burton, N., Christiansen, J., Feng, G., Hill, B., Houghton, D., Kaufman, M., Rao, J., Sharpe, J., Ross, A., Stevenson, P., Venkataraman, S., Waterhouse, A., Yang, Y. and Davidson, D.R., 2003. EMAP and EMAGE: a framework for understanding spatially organized data. Neuroinformatics. 1, 309-25.

Ballycuif, L., Alvaradomallart, R.M., Darnell, D.K. and Wassef, M., 1992. Relationship between Wnt-1 and En-2 Expression Domains during Early Development of Normal and Ectopic Met-Mesencephalon. Development. 115, 999-1009.

Barth, K.A., Kishimoto, Y., Rohr, K.B., Seydler, C., Schulte-Merker, S. and Wilson, S.W., 1999. Bmp activity establishes a gradient of positional information throughout the entire neural plate. Development. 126, 4977-4987.

Basile, J.R., Afkhami, T. and Gutkind, J.S., 2005. Semaphorin 4D/plexin-B1 induces endothelial cell migration through the activation of PYK2, Src, and the phosphatidylinositol 3-kinase-Akt pathway. Mol Cell Biol. 25, 6889-98.

Beckers, A., Alten, L., Viebahn, C., Andre, P. and Gossler, A., 2007. The mouse homeobox gene Noto regulates node morphogenesis, notochordal ciliogenesis, and left right patterning. Proc Natl Acad Sci U S A. 104, 15765-70.

Beddington, R.S., 1994. Induction of a second neural axis by the mouse node. Development. 120, 613-20.

Beddington, R.S.P. and Robertson, E.J., 1999. Axis development and early asymmetry in mammals. Cell. 96, 195-209.

Bellairs, R., 1954. The effects of tetra-sodium 2-methyl 1:4-naphthohydroquinone diphosphate on early chick and amphibian embryos. Br J Cancer. 8, 685-92.

Belo, J.A., Silva, A.C., Borges, A.C., Filipe, M., Bento, M., Goncalves, L., Vitorino, M., Salgueiro, A.M., Texeira, V., Tavares, A.T. and Marques, S., 2009. Generating asymmetries in the early vertebrate embryo: the role of the Cerberus-like family. Int J Dev Biol. 53, 1399-407.

Bensoussan-Trigano, V., Lallemand, Y., Saint Cloment, C. and Robert, B., 2011. Msx1 and Msx2 in limb mesenchyme modulate digit number and identity. Dev Dyn. 240, 1190-202.

Bhattacharyya, S., Bailey, A.P., Bronner-Fraser, M. and Streit, A., 2004. Segregation of lens and olfactory precursors from a common territory: cell sorting and reciprocity of Dlx5 and Pax6 expression. Dev Biol. 271, 403-14.

Bi, L., Okabe, I., Bernard, D.J. and Nussbaum, R.L., 2002. Early embryonic lethality in mice deficient in the p110beta catalytic subunit of PI 3-kinase. Mamm Genome. 13, 169-72.

Blum, M., Gaunt, S.J., Cho, K.W.Y., Steinbeisser, H., Blumberg, B., Bittner, D. and Derobertis, E.M., 1992a. Gastrulation in the Mouse - the Role of the Homeobox Gene Goosecoid. Cell. 69, 1097-1106.

Blum, M., Gaunt, S.J. and Derobertis, E.M., 1992b. Gastrulation in the Mouse - the Role of the Homeobox Gene Goosecoid. Molecular Biology of the Cell. 3, A98-A98.

Bornstein, P., 1995. Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. J Cell Biol. 130, 503-6.

Bornstein, P. and Sage, E.H., 2002. Matricellular proteins: extracellular modulators of cell function. Curr Opin Cell Biol. 14, 608-16.

Bouwmeester, T., Kim, S.H., Sasai, Y., Lu, B. and DeRobertis, E.M., 1996. Cerberus is a head-inducing secreted factor expressed in the anterior endoderm of Spemann's organizer. Nature. 382, 595-601.

Brault, V., Moore, R., Kutsch, S., Ishibashi, M., Rowitch, D.H., McMahon, A.P., Sommer, L., Boussadia, O. and Kemler, R., 2001. Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. Development. 128, 1253-64.

Braun, M., Wunderlin, M., Spieth, K., Knochel, W., Gierschik, P. and Moepps, B., 2002. Xenopus laevis Stromal cell-derived factor 1: conservation of structure and function during vertebrate development. J Immunol. 168, 2340-7.

Braun, M.M., Etheridge, A., Bernard, A., Robertson, C.P. and Roelink, H., 2003. Wnt signaling is required at distinct stages of development for the induction of the posterior forebrain. Development. 130, 5579-5587.

Brenner, R., Perez, G.J., Bonev, A.D., Eckman, D.M., Kosek, J.C., Wiler, S.W., Patterson, A.J., Nelson, M.T. and Aldrich, R.W., 2000. Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. Nature. 407, 870-6.

Briscoe, J., Chen, Y., Jessell, T.M. and Struhl, G., 2001. A hedgehog-insensitive form of patched provides evidence for direct long-range morphogen activity of Sonic hedgehog in the neural tube. Mol Cell. 7, 1279-1291.

Briscoe, J. and Ericson, J., 2001. Specification of neuronal fates in the ventral neural tube. Current Opinion in Neurobiology. 11, 43-49.

Briscoe, J., Pierani, A., Jessell, T.M. and Ericson, J., 2000. A homeodomain protein code specifies progenitor cell identity and neuronal fate in the ventral neural tube. Cell. 101, 435-445.

Broccoli, V., Boncinelli, E. and Wurst, W., 1999. The caudal limit of Otx2 expression positions the isthmic organizer. Nature. 401, 164-168.

Brugger, S.M., Merrill, A.E., Torres-Vazquez, J., Wu, N., Ting, M.C., Cho, J.Y., Dobias, S.L., Yi, S.E., Lyons, K., Bell, J.R., Arora, K., Warrior, R. and Maxson, R., 2004. A phylogenetically conserved cis-regulatory module in the Msx2 promoter is

sufficient for BMP-dependent transcription in murine and Drosophila embryos. Development. 131, 5153-65.
Butler, D.C., Haramizu, S., Williamson, D.L. and Alway, S.E., 2009. Phospho-ablated Id2 is growth suppressive and pro-apoptotic in proliferating myoblasts. PLoS One. 4, e6302.
Butros, J., 1967. Limited Axial Structures in Nodeless Chick Blastoderms. J Embryol Exp Morphol. 17, 119-&.
Calvo, J. and Boya, J., 1978. Embryonic development of the pineal gland of the chicken (Gallus gallus). Acta Anat (Basel). 101, 289-303.
Calvo, J. and Boya, J., 1979. Ultrastructural study of the embryonic development of the pineal gland of the chicken (Gallus gallus). Acta Anat (Basel). 103, 39-73.
Campione, M., Steinbeisser, H., Schweickert, A., Deissler, K., van Bebber, F., Lowe, L.A., Nowotschin, S., Viebahn, C., Haffter, P., Kuehn, M.R. and Blum, M., 1999. The homeobox gene Pitx2: mediator of asymmetric left-right signaling in vertebrate heart and gut looping. Development. 126, 1225-34.
Capdevila, J. and Izpisua Belmonte, J.C., 2001. Patterning mechanisms controlling vertebrate limb development. Annu Rev Cell Dev Biol. 17, 87-132.
Capdevila, J., Vogan, K.J., Tabin, C.J. and Izpisua Belmonte, J.C., 2000. Mechanisms of left-right determination in vertebrates. Cell. 101, 9-21.
Catala, M., Teillet, M.A., DeRobertis, E.M. and LeDouarin, N.M., 1996. A spinal cord fate map in the avian embryo: While regressing, Hensen's node lays down the notochord and floor plate thus joining the spinal cord lateral walls. Development. 122, 2599-2610.
Chambers, D., Wilson, L., Maden, M. and Lumsden, A., 2007. RALDH-independent generation of retinoic acid during vertebrate embryogenesis by CYP1B1. Development. 134, 1369-83.
Chang, D.T., Lopez, A., von Kessler, D.P., Chiang, C., Simandl, B.K., Zhao, R., Seldin, M.F., Fallon, J.F. and Beachy, P.A., 1994. Products, genetic linkage and limb patterning activity of a murine hedgehog gene. Development. 120, 3339-53.
Chen, J.N., vanEeden, F.J.M., Warren, K.S., Chin, A., NussleinVolhard, C., Haffter, P. and Fishman, M.C., 1997. Left-right pattern of cardiac BMP4 may drive asymmetry of the heart in zebrafish. Development. 124, 4373-4382.
Chiang, C., Litingtung, Y., Lee, E., Young, K.E., Corden, J.L., Westphal, H. and Beachy, P.A., 1996. Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. Nature. 383, 407-13.
Chilov, D., Sinjushina, N., Saarimaki-Vire, J., Taketo, M.M. and Partanen, J., 2010. beta-Catenin regulates intercellular signalling networks and cell-type specific transcription in the developing mouse midbrain-rhombomere 1 region. PLoS One. 5, e10881.
Cho, K.W., Blumberg, B., Steinbeisser, H. and De Robertis, E.M., 1991. Molecular nature of Spemann's organizer: the role of the Xenopus homeobox gene goosecoid. Cell. 67, 1111-20.
Christensen, C., Zhang, S. and Roelink, H., 2006. Inhibition of cGMP-dependent protein kinase reduces the response to sonic hedgehog in neuralized embryoid bodies. Stem Cells Dev. 15, 647-54.
Chuang, P.T. and McMahon, A.P., 1999. Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. Nature. 397, 617-621.
Coelho, C.N., Sumoy, L., Rodgers, B.J., Davidson, D.R., Hill, R.E., Upholt, W.B. and Kosher, R.A., 1991. Expression of the chicken homeobox-containing gene GHox-8 during embryonic chick limb development. Mech Dev. 34, 143-54.

Collignon, J., Varlet, I. and Robertson, E.J., 1996. Relationship between asymmetric nodal expression and the direction of embryonic turning. Nature. 381, 155-8.
Collins, S.P. and Uhler, M.D., 1997. Characterization of PKIgamma, a novel isoform of the protein kinase inhibitor of cAMP-dependent protein kinase. J Biol Chem. 272, 18169-78.
Concha, M.L. and Wilson, S.W., 2001. Asymmetry in the epithalamus of vertebrates. J Anat. 199, 63-84.
Constam, D.B. and Robertson, E.J., 2000. SPC4/PACE4 regulates a TGFbeta signaling network during axis formation. Genes Dev. 14, 1146-55.
Cooper, M.A., Son, A.I., Komlos, D., Sun, Y., Kleiman, N.J. and Zhou, R., 2008. Loss of ephrin-A5 function disrupts lens fiber cell packing and leads to cataract. Proc Natl Acad Sci U S A. 105, 16620-5.
Corbeil, D., Marzesco, A.M., Wilsch-Brauninger, M. and Huttner, W.B., 2010. The intriguing links between prominin-1 (CD133), cholesterol-based membrane microdomains, remodeling of apical plasma membrane protrusions, extracellular membrane particles, and (neuro)epithelial cell differentiation. FEBS Lett. 584, 1659-64.
Couly, G.F. and Le Douarin, N.M., 1985. Mapping of the early neural primordium in quail-chick chimeras. I. Developmental relationships between placodes, facial ectoderm, and prosencephalon. Dev Biol. 110, 422-39.
Couly, G.F. and Le Douarin, N.M., 1987. Mapping of the early neural primordium in quail-chick chimeras. II. The prosencephalic neural plate and neural folds: implications for the genesis of cephalic human congenital abnormalities. Dev Biol. 120, 198-214.
Creemers, J.W., Groot Kormelink, P.J., Roebroek, A.J., Nakayama, K. and Van de Ven, W.J., 1993. Proprotein processing activity and cleavage site selectivity of the Kex2-like endoprotease PACE4. FEBS Lett. 336, 65-9.
Crossley, P.H., Martinez, S., Ohkubo, Y. and Rubenstein, J.L., 2001. Coordinate expression of Fgf8, Otx2, Bmp4, and Shh in the rostral prosencephalon during development of the telencephalic and optic vesicles. Neuroscience. 108, 183-206.
Csernus, V.J., Nagy, A.D. and Faluhelyi, N., 2007. Development of the rhythmic melatonin secretion in the embryonic chicken pineal gland. Gen Comp Endocrinol. 152, 148-53.
Cui, C., Little, C.D. and Rongish, B.J., 2009a. Rotation of organizer tissue contributes to left-right asymmetry. Anat Rec (Hoboken). 292, 557-61.
Cui, J., Yang, H. and Lee, U.S., 2009b. Molecular mechanisms of BK channel activation. Cell Mol Life Sci. 66, 852-75.
Dale, K., Sattar, N., Heemskerk, J., Clarke, J.D.W., Placzek, M. and Dodd, J., 1999. Differential patterning of ventral midline cells by axial mesoderm is regulated by BMP7 and chordin. Development. 126, 397-408.
Davidson, B.P., Kinder, S.J., Steiner, K., Schoenwolf, G.C. and Tam, P.P., 1999. Impact of node ablation on the morphogenesis of the body axis and the lateral asymmetry of the mouse embryo during early organogenesis. Dev Biol. 211, 11-26.
De Robertis, E.M., Larrain, J., Oelgeschlager, M. and Wessely, O., 2000. The establishment of Spemann's organizer and patterning of the vertebrate embryo. Nat Rev Genet. 1, 171-81.
De Robertis, E.M., Wessely, O., Oelgeschlager, M., Brizuela, B., Pera, E., Larrain, J., Abreu, J. and Bachiller, D., 2001. Molecular mechanisms of cell-cell signaling by the Spemann-Mangold organizer. Int J Dev Biol. 45, 189-97.
de Vega, S., Iwamoto, T., Nakamura, T., Hozumi, K., McKnight, D.A., Fisher, L.W., Fukumoto, S. and Yamada, Y., 2007. TM14 is a new member of the fibulin family

(fibulin-7) that interacts with extracellular matrix molecules and is active for cell binding. J Biol Chem. 282, 30878-88.
Dedeic, Z., Cetera, M., Cohen, T.V. and Holaska, J.M., 2011. Emerin inhibits Lmo7 binding to the Pax3 and MyoD promoters and expression of myoblast proliferation genes. J Cell Sci. 124, 1691-702.
Dessaud, E., McMahon, A.P. and Briscoe, J., 2008. Pattern formation in the vertebrate neural tube: a sonic hedgehog morphogen-regulated transcriptional network. Development. 135, 2489-503.
Dey, B.R., Spence, S.L., Nissley, P. and Furlanetto, R.W., 1998. Interaction of human suppressor of cytokine signaling (SOCS)-2 with the insulin-like growth factor-I receptor. J Biol Chem. 273, 24095-101.
Dias, M.S. and Schoenwolf, G.C., 1990. Formation of ectopic neurepithelium in chick blastoderms: age-related capacities for induction and self-differentiation following transplantation of quail Hensen's nodes. Anat Rec. 228, 437-48.
Diez-Alarcia, R., Mostany, R., Dos-Anjos, S. and Fernandez-Lopez, A., 2009. Functional autoradiography and gene expression analysis applied to the characterization of the alpha2-adrenergic system in the chicken brain. J Chem Neuroanat. 38, 282-91.
Ding, Q., Motoyama, J., Gasca, S., Mo, R., Sasaki, H., Rossant, J. and Hui, C.C., 1998. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. Development. 125, 2533-43.
Dosch, R., Gawantka, V., Delius, H., Blumenstock, C. and Niehrs, C., 1997. Bmp-4 acts as a morphogen in dorsoventral mesoderm patterning in Xenopus. Development. 124, 2325-34.
Doskeland, S.O., Maronde, E. and Gjertsen, B.T., 1993. The genetic subtypes of cAMP-dependent protein kinase--functionally different or redundant? Biochim Biophys Acta. 1178, 249-58.
Drew, C.F., Lin, C.M., Jiang, T.X., Blunt, G., Mou, C., Chuong, C.M. and Headon, D.J., 2007. The Edar subfamily in feather placode formation. Dev Biol. 305, 232-45.
Eagleson, G.W. and Dempewolf, R.D., 2002. The role of the anterior neural ridge and Fgf-8 in early forebrain patterning and regionalization in Xenopus laevis. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology. 132, 179-189.
Eagleson, G.W. and Harris, W.A., 1990. Mapping of the presumptive brain regions in the neural plate of Xenopus laevis. J Neurobiol. 21, 427-40.
Eberhart, J., Swartz, M., Koblar, S.A., Pasquale, E.B., Tanaka, H. and Krull, C.E., 2000. Expression of EphA4, ephrin-A2 and ephrin-A5 during axon outgrowth to the hindlimb indicates potential roles in pathfinding. Dev Neurosci. 22, 237-50.
Echelard, Y., Epstein, D.J., Stjacques, B., Shen, L., Mohler, J., Mcmahon, J.A. and Mcmahon, A.P., 1993. Sonic-Hedgehog, a Member of a Family of Putative Signaling Molecules, Is Implicated in the Regulation of Cns Polarity. Cell. 75, 1417-1430.
Echevarria, D., Vieira, C., Gimeno, L. and Martinez, S., 2003. Neuroepithelial secondary organizers and cell fate specification in the developing brain. Brain Res Brain Res Rev. 43, 179-91.
Ekker, M., Akimenko, M.A., Bremiller, R. and Westerfield, M., 1992. Regional expression of three homeobox transcripts in the inner ear of zebrafish embryos. Neuron. 9, 27-35.
Ellies, D.L., Stock, D.W., Hatch, G., Giroux, G., Weiss, K.M. and Ekker, M., 1997. Relationship between the genomic organization and the overlapping embryonic expression patterns of the zebrafish dlx genes. Genomics. 45, 580-90.
Ericson, J., Morton, S., Kawakami, A., Roelink, H. and Jessell, T.M., 1996. Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. Cell. 87, 661-73.

Ericson, J., Muhr, J., Placzek, M., Lints, T., Jessell, T.M. and Edlund, T., 1995. Sonic Hedgehog Induces the Differentiation of Ventral Forebrain Neurons - a Common Signal for Ventral Patterning within the Neural-Tube. Cell. 81, 747-756.

Ernsberger, U., Reissmann, E., Mason, I. and Rohrer, H., 2000. The expression of dopamine beta-hydroxylase, tyrosine hydroxylase, and Phox2 transcription factors in sympathetic neurons: evidence for common regulation during noradrenergic induction and diverging regulation later in development. Mech Dev. 92, 169-77.

Essner, J.J., Amack, J.D., Nyholm, M.K., Harris, E.B. and Yost, J., 2005. Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. Development. 132, 1247-1260.

Eyal-Giladi, H. and Kochav, S., 1976. From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. Dev Biol. 49, 321-37.

Fainsod, A., Deissler, K., Yelin, R., Marom, K., Epstein, M., Pillemer, G., Steinbeisser, H. and Blum, M., 1997. The dorsalizing and neural inducing gene follistatin is an antagonist of BMP-4. Mech Dev. 63, 39-50.

Fasciotto, B.H., Trauss, C.A., Greeley, G.H. and Cohn, D.V., 1993. Parastatin (porcine chromogranin A347-419), a novel chromogranin A-derived peptide, inhibits parathyroid cell secretion. Endocrinology. 133, 461-6.

Feldheim, D.A. and O'Leary, D.D., 2010. Visual map development: bidirectional signaling, bifunctional guidance molecules, and competition. Cold Spring Harb Perspect Biol. 2, a001768.

Fernandez-Lopez, A., del Arco, C., Gonzalez, A.M., Gomez, T., Calvo, P. and Pazos, A., 1990. Autoradiographic localization of alpha 2-adrenoceptors in chick brain. Neurosci Lett. 120, 97-100.

Figdor, M.C. and Stern, C.D., 1993. Segmental organization of embryonic diencephalon. Nature. 363, 630-4.

Francis, S.H. and Corbin, J.D., 1994. Structure and function of cyclic nucleotide-dependent protein kinases. Annu Rev Physiol. 56, 237-72.

Furuta, Y., Piston, D.W. and Hogan, B.L.M., 1997. Bone morphogenetic proteins (BMPs) as regulators of dorsal forebrain development. Development. 124, 2203-2212.

Gall, J.G. and Pardue, M.L., 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc Natl Acad Sci U S A. 63, 378-83.

Gallera, J., 1970. [The action of actinomycin D on the inductive capacity of Hensen's node and the neuralization of chick ectoderm]. J Embryol Exp Morphol. 23, 473-89.

Gallera, J. and Ivanov, I., 1964. [Neurogenic Competence of the External Layer of the Chick Blastoderm as a Function of the 'Time' Factor]. J Embryol Exp Morphol. 12, 693-711.

Gallicano, G.I., Bauer, C. and Fuchs, E., 2001. Rescuing desmoplakin function in extra-embryonic ectoderm reveals the importance of this protein in embryonic heart, neuroepithelium, skin and vasculature. Development. 128, 929-41.

Gallicano, G.I., Kouklis, P., Bauer, C., Yin, M., Vasioukhin, V., Degenstein, L. and Fuchs, E., 1998. Desmoplakin is required early in development for assembly of desmosomes and cytoskeletal linkage. J Cell Biol. 143, 2009-22.

Gamse, J.T., Thisse, C., Thisse, B. and Halpern, M.E., 2003. The parapineal mediates left-right asymmetry in the zebrafish diencephalon. Development. 130, 1059-68.

Garcia-Frigola, C., Burgaya, F., de Lecea, L. and Soriano, E., 2001. Pattern of expression of the tetraspanin Tspan-5 during brain development in the mouse. Mech Dev. 106, 207-12.

Garcia-Gras, E., Lombardi, R., Giocondo, M.J., Willerson, J.T., Schneider, M.D., Khoury, D.S. and Marian, A.J., 2006. Suppression of canonical Wnt/beta-catenin signaling by

nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. J Clin Invest. 116, 2012-21.
Garcia-Lopez, R., Vieira, C., Echevarria, D. and Martinez, S., 2004. Fate map of the diencephalon and the zona limitans at the 10-somites stage in chick embryos. Dev Biol. 268, 514-30.
Gawantka, V., Pollet, N., Delius, H., Vingron, M., Pfister, R., Nitsch, R., Blumenstock, C. and Niehrs, C., 1998. Gene expression screening in Xenopus identifies molecular pathways, predicts gene function and provides a global view of embryonic patterning. Mech Dev. 77, 95-141.
Geering, B., Cutillas, P.R., Nock, G., Gharbi, S.I. and Vanhaesebroeck, B., 2007. Class IA phosphoinositide 3-kinases are obligate p85-p110 heterodimers. Proc Natl Acad Sci U S A. 104, 7809-14.
Gerke, P., Benzing, T., Hohne, M., Kispert, A., Frotscher, M., Walz, G. and Kretz, O., 2006. Neuronal expression and interaction with the synaptic protein CASK suggest a role for Neph1 and Neph2 in synaptogenesis. J Comp Neurol. 498, 466-75.
Glinka, A., Wu, W., Delius, H., Monaghan, A.P., Blumenstock, C. and Niehrs, C., 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature. 391, 357-362.
Gont, L.K., Steinbeisser, H., Blumberg, B. and de Robertis, E.M., 1993. Tail formation as a continuation of gastrulation: the multiple cell populations of the Xenopus tailbud derive from the late blastopore lip. Development. 119, 991-1004.
Goodrich, L.V., Johnson, R.L., Milenkovic, L., McMahon, J.A. and Scott, M.P., 1996. Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by Hedgehog. Genes Dev. 10, 301-12.
Grabowski, C.T., 1956. The effects of the excision of Hansen's node on the early development of the chick embryo. J. Exp. Zool. 133, 301-344.
Grapin-Botton, A., Cambronero, F., Weiner, H.L., Bonnin, M.A., Puelles, L. and Le Douarin, N.M., 1999. Patterning signals acting in the spinal cord override the organizing activity of the isthmus. Mech Dev. 84, 41-53.
Gu, W.X. and Kania, A., 2010. Identification of genes controlled by LMX1B in E13.5 mouse limbs. Dev Dyn. 239, 2246-55.
Guinazu, M.F., Chambers, D., Lumsden, A. and Kiecker, C., 2007. Tissue interactions in the developing chick diencephalon. Neural Dev. 2, 25.
Gulacsi, A.A. and Anderson, S.A., 2008. beta-catenin-mediated Wnt signaling regulates neurogenesis in the ventral telencephalon. Nat Neurosci. 11, 1383-1391.
Gunhaga, L., Marklund, M., Sjodal, M., Hsieh, J.C., Jessell, T.M. and Edlund, T., 2003. Specification of dorsal telencephalic character by sequential Wnt and FGF signaling. Nat Neurosci. 6, 701-707.
Guttikar, G., Patil, S., Goel, C., Rane, R.K., Ghatpande, S.K., Joshi, M.V., Mulherkar, L. and Modak, S.P., 1993. Multiple neural inductions in area opaca by grafts of the Hensen's node do not retard host chick embryo development. Indian J Exp Biol. 31, 803-7.
Halpern, M.E., Ho, R.K., Walker, C. and Kimmel, C.B., 1993. Induction of Muscle Pioneers and Floor Plate Is Distinguished by the Zebrafish No Tail Mutation. Cell. 75, 99-111.
Halpern, M.E., Thisse, C., Ho, R.K., Thisse, B., Riggleman, B., Trevarrow, B., Weinberg, E.S., Postlethwait, J.H. and Kimmel, C.B., 1995. Cell-autonomous shift from axial to paraxial mesodermal development in zebrafish floating head mutants. Development. 121, 4257-4264.
Hamburger, V. and Hamilton, H.L., 1951. A series of normal stages in the development of the chick embryo. Journal of Morphology. 88, 49-92.

Hamburger, V. and Hamilton, H.L., 1992. A series of normal stages in the development of the chick embryo. 1951. Dev Dyn. 195, 231-72.
Hansen, C.S., Marion, C.D., Steele, K., George, S. and Smith, W.C., 1997. Direct neural induction and selective inhibition of mesoderm and epidermis inducers by Xnr3. Development. 124, 483-492.
Hashimoto-Torii, K., Motoyama, J., Hui, C.C., Kuroiwa, A., Nakafuku, M. and Shimamura, K., 2003. Differential activities of Sonic hedgehog mediated by Gli transcription factors define distinct neuronal subtypes in the dorsal thalamus. Mech Dev. 120, 1097-111.
Hashimoto, T., Schlessinger, D. and Cui, C.Y., 2008. Troy binding to lymphotoxin-alpha activates NF kappa B mediated transcription. Cell Cycle. 7, 106-11.
Hatta, K. and Takahashi, Y., 1996. Secondary axis induction by heterospecific organizers in zebrafish. Developmental Dynamics. 205, 183-195.
He, Z. and Tessier-Lavigne, M., 1997. Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. Cell. 90, 739-51.
Hemler, M.E., 2005. Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol. 6, 801-11.
Hemmati-Brivanlou, A. and Melton, D., 1997. Vertebrate embryonic cells will become nerve cells unless told otherwise. Cell. 88, 13-7.
Hemmatibrivanlou, A., Kelly, O.G. and Melton, D.A., 1994. Follistatin, an Antagonist of Activin, Is Expressed in the Spemann Organizer and Displays Direct Neuralizing Activity. Cell. 77, 283-295.
Hemmatibrivanlou, A. and Melton, D.A., 1994. Inhibition of Activin Receptor Signaling Promotes Neuralization in Xenopus. Cell. 77, 273-281.
Hensen, V., 1875. Uber ein neues Strukturverhaltnis der quergestreiften muskelfase. Arb Physiol. Inst. Kiel. 1868, 1-26.
Herman, S.E. and Johnson, A.J., 2012. Molecular pathways: targeting phosphoinositide 3-kinase p110-delta in chronic lymphocytic leukemia. Clin Cancer Res. 18, 4013-8.
Hidalgo-Sanchez, M., Millet, S., Simeone, A. and Alvarado-Mallart, R.M., 1999. Comparative analysis of Otx2, Gbx2, Pax2, Fgf8 and Wnt1 gene expressions during the formation of the chick midbrain/hindbrain domain. Mech Dev. 81, 175-8.
Hikosaka, O., Sesack, S.R., Lecourtier, L. and Shepard, P.D., 2008. Habenula: crossroad between the basal ganglia and the limbic system. J Neurosci. 28, 11825-9.
Hill, R.E., Jones, P.F., Rees, A.R., Sime, C.M., Justice, M.J., Copeland, N.G., Jenkins, N.A., Graham, E. and Davidson, D.R., 1989. A new family of mouse homeo box-containing genes: molecular structure, chromosomal location, and developmental expression of Hox-7.1. Genes Dev. 3, 26-37.
Hilton, D.J., Richardson, R.T., Alexander, W.S., Viney, E.M., Willson, T.A., Sprigg, N.S., Starr, R., Nicholson, S.E., Metcalf, D. and Nicola, N.A., 1998. Twenty proteins containing a C-terminal SOCS box form five structural classes. Proc Natl Acad Sci U S A. 95, 114-9.
Hirai, H., Pang, Z., Bao, D., Miyazaki, T., Li, L., Miura, E., Parris, J., Rong, Y., Watanabe, M., Yuzaki, M. and Morgan, J.I., 2005. Cbln1 is essential for synaptic integrity and plasticity in the cerebellum. Nat Neurosci. 8, 1534-41.
Hirata, T., Takagi, S. and Fujisawa, H., 1993. The membrane protein A5, a putative neuronal recognition molecule, promotes neurite outgrowth. Neurosci Res. 17, 159-69.
Ho, R.K. and Kimmel, C.B., 1993. Commitment of cell fate in the early zebrafish embryo. Science. 261, 109-11.
Hofmann, F., Bernhard, D., Lukowski, R. and Weinmeister, P., 2009. cGMP regulated protein kinases (cGK). Handb Exp Pharmacol. 137-62.

Hogan, B.L., Thaller, C. and Eichele, G., 1992. Evidence that Hensen's node is a site of retinoic acid synthesis. Nature. 359, 237-41.
Holaska, J.M., Rais-Bahrami, S. and Wilson, K.L., 2006. Lmo7 is an emerin-binding protein that regulates the transcription of emerin and many other muscle-relevant genes. Hum Mol Genet. 15, 3459-72.
Holley, S.A., Jackson, P.D., Sasai, Y., Lu, B., De Robertis, E.M., Hoffmann, F.M. and Ferguson, E.L., 1995. A conserved system for dorsal-ventral patterning in insects and vertebrates involving sog and chordin. Nature. 376, 249-53.
Hongo, I., Kengaku, M. and Okamoto, H., 1999. FGF signaling and the anterior neural induction in Xenopus. Dev Biol. 216, 561-581.
Horii, Y., Beeler, J.F., Sakaguchi, K., Tachibana, M. and Miki, T., 1994. A novel oncogene, ost, encodes a guanine nucleotide exchange factor that potentially links Rho and Rac signaling pathways. EMBO J. 13, 4776-86.
Hornbruch, A. and Wolpert, L., 1986. Positional signalling by Hensen's node when grafted to the chick limb bud. J Embryol Exp Morphol. 94, 257-65.
Houart, C., Westerfield, M. and Wilson, S.W., 1998a. A small population of anterior cells patterns for forebrain during zebrafish gastrulation. Nature. 391, 788-792.
Houart, C., Westerfield, M. and Wilson, S.W., 1998b. A small population of anterior cells patterns the forebrain during zebrafish gastrulation. Nature. 391, 788-92.
Hu, S., Tamada, K., Ni, J., Vincenz, C. and Chen, L., 1999. Characterization of TNFRSF19, a novel member of the tumor necrosis factor receptor superfamily. Genomics. 62, 103-7.
Iacangelo, A.L. and Eiden, L.E., 1995. Chromogranin A: current status as a precursor for bioactive peptides and a granulogenic/sorting factor in the regulated secretory pathway. Regul Pept. 58, 65-88.
Ida, M., Suzuki, H., Mori, N., Taketani, S. and Araki, M., 2004. Neuro-p24 plays an essential role in neurite extension: antisense oligonucleotide inhibition of neurite extension in cultured DRG neurons and neuroblastoma cells. Neurosci Res. 50, 199-208.
Ikeno, T., Hashimoto, S., Kuzuya, H. and Nagatsu, T., 1977. Purification and properties of human serum dopamine-beta-hydroxylase. Mol Cell Biochem. 18, 117-23.
Inoue, T., Nakamura, S. and Osumi, N., 2000. Fate mapping of the mouse prosencephalic neural plate. Dev Biol. 219, 373-83.
Ishibashi, M. and McMahon, A.P., 2002. A sonic hedgehog-dependent signaling relay regulates growth of diencephalic and mesencephalic primordia in the early mouse embryo. Development. 129, 4807-4819.
Izpisua-Belmonte, J.C., De Robertis, E.M., Storey, K.G. and Stern, C.D., 1993. The homeobox gene goosecoid and the origin of organizer cells in the early chick blastoderm. Cell. 74, 645-59.
Jaumann, M., Dettling, J., Gubelt, M., Zimmermann, U., Gerling, A., Paquet-Durand, F., Feil, S., Wolpert, S., Franz, C., Varakina, K., Xiong, H., Brandt, N., Kuhn, S., Geisler, H.S., Rohbock, K., Ruth, P., Schlossmann, J., Hutter, J., Sandner, P., Feil, R., Engel, J., Knipper, M. and Ruttiger, L., 2012. cGMP-Prkg1 signaling and Pde5 inhibition shelter cochlear hair cells and hearing function. Nat Med. 18, 252-9.
Jessell, T.M., 2000. Neuronal specification in the spinal cord: Inductive signals and transcriptional codes. Nature Reviews Genetics. 1, 20-29.
Johnson, R.L. and Tabin, C., 1995. The long and short of hedgehog signaling. Cell. 81, 313-6.
Jou, S.T., Carpino, N., Takahashi, Y., Piekorz, R., Chao, J.R., Carpino, N., Wang, D. and Ihle, J.N., 2002. Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex. Mol Cell Biol. 22, 8580-91.

Joubin, K. and Stern, C.D., 1999. Molecular interactions continuously define the organizer during the cell movements of gastrulation. Cell. 98, 559-571.
Joyner, A.L., 1996. Engrailed, Wnt and Pax genes regulate midbrain--hindbrain development. Trends Genet. 12, 15-20.
Kadota, Y., Niiya, A., Masaki, R., Yamamoto, A., Araki, M. and Taketani, S., 1997. A newly identified membrane protein localized exclusively in intracellular organelles of neurons. Brain Res Mol Brain Res. 46, 265-73.
Kamaid, A. and Giraldez, F., 2008. Btg1 and Btg2 gene expression during early chick development. Dev Dyn. 237, 2158-69.
Kambe, T., Suzuki, T., Nagao, M. and Yamaguchi-Iwai, Y., 2006. Sequence similarity and functional relationship among eukaryotic ZIP and CDF transporters. Genomics Proteomics Bioinformatics. 4, 1-9.
Kambe, T., Yamaguchi-Iwai, Y., Sasaki, R. and Nagao, M., 2004. Overview of mammalian zinc transporters. Cell Mol Life Sci. 61, 49-68.
Kameda, Y., Miura, M. and Ohno, S., 1998. Localization and development of chromogranin A and luteinizing hormone immunoreactivities in the secretory-specific cells of the hypophyseal pars tuberalis of the chicken. Histochem Cell Biol. 109, 211-22.
Katahira, T., Sato, T., Sugiyama, S., Okafuji, T., Araki, I., Funahashi, J. and Nakamura, H., 2000. Interaction between Otx2 and Gbx2 defines the organizing center for the optic tectum. Mech Dev. 91, 43-52.
Kavety, B., Jenkins, N.A., Fletcher, C.F., Copeland, N.G. and Morgan, J.I., 1994. Genomic structure and mapping of precerebellin and a precerebellin-related gene. Brain Res Mol Brain Res. 27, 152-6.
Kawakami, M. and Nakanishi, N., 2001. The role of an endogenous PKA inhibitor, PKIalpha, in organizing left-right axis formation. Development. 128, 2509-15.
Kawakami, Y., Raya, A., Raya, R.M., Rodriguez-Esteban, C. and Belmonte, J.C.I., 2005. Retinoic acid signalling links left-right asymmetric patterning and bilaterally symmetric somitogenesis in the zebrafish embryo. Nature. 435, 165-171.
Kawano, Y. and Kypta, R., 2003. Secreted antagonists of the Wnt signalling pathway. J Cell Sci. 116, 2627-34.
Kawasaki, T., Kitsukawa, T., Bekku, Y., Matsuda, Y., Sanbo, M., Yagi, T. and Fujisawa, H., 1999. A requirement for neuropilin-1 in embryonic vessel formation. Development. 126, 4895-902.
Khokha, M.K., Hsu, D., Brunet, L.J., Dionne, M.S. and Harland, R.M., 2003. Gremlin is the BMP antagonist required for maintenance of Shh and Fgf signals during limb patterning. Nat Genet. 34, 303-7.
Kiecker, C. and Lumsden, A., 2004. Hedgehog signaling from the ZLI regulates diencephalic regional identity. Nat Neurosci. 7, 1242-9.
Kimmel, C.B., Warga, R.M. and Schilling, T.F., 1990. Origin and organization of the zebrafish fate map. Development. 108, 581-94.
Kimura, I., Konishi, M., Miyake, A., Fujimoto, M. and Itoh, N., 2006. Neudesin, a secreted factor, promotes neural cell proliferation and neuronal differentiation in mouse neural precursor cells. J Neurosci Res. 83, 1415-24.
Kintner, C.R. and Dodd, J., 1991. Hensen Node Induces Neural Tissue in Xenopus Ectoderm - Implications for the Action of the Organizer in Neural Induction. Development. 113, 1495-1505.
Kitsukawa, T., Shimono, A., Kawakami, A., Kondoh, H. and Fujisawa, H., 1995. Overexpression of a membrane protein, neuropilin, in chimeric mice causes anomalies in the cardiovascular system, nervous system and limbs. Development. 121, 4309-18.

Knecht, A.K. and Bronner-Fraser, M., 2001. DBHR, a gene with homology to dopamine beta-hydroxylase, is expressed in the neural crest throughout early development. Dev Biol. 234, 365-75.
Koeslag, J.H., Saunders, P.T. and Wessels, J.A., 1999. The chromogranins and the counter-regulatory hormones: do they make homeostatic sense? J Physiol. 517 (Pt 3), 643-9.
Kohtz, J.D., Baker, D.P., Corte, G. and Fishell, G., 1998. Regionalization within the mammalian telencephalon is mediated by changes in responsiveness to Sonic Hedgehog. Development. 125, 5079-89.
Kojima, T., Morikawa, Y., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Senba, E. and Kitamura, T., 2000. TROY, a newly identified member of the tumor necrosis factor receptor superfamily, exhibits a homology with Edar and is expressed in embryonic skin and hair follicles. J Biol Chem. 275, 20742-7.
Kok, K., Nock, G.E., Verrall, E.A., Mitchell, M.P., Hommes, D.W., Peppelenbosch, M.P. and Vanhaesebroeck, B., 2009. Regulation of p110delta PI 3-kinase gene expression. PLoS One. 4, e5145.
Kolodkin, A.L., Levengood, D.V., Rowe, E.G., Tai, Y.T., Giger, R.J. and Ginty, D.D., 1997. Neuropilin is a semaphorin III receptor. Cell. 90, 753-62.
Kowalczyk, A.P., Bornslaeger, E.A., Norvell, S.M., Palka, H.L. and Green, K.J., 1999. Desmosomes: intercellular adhesive junctions specialized for attachment of intermediate filaments. Int Rev Cytol. 185, 237-302.
Kozlowski, D.J., Murakami, T., Ho, R.K. and Weinberg, E.S., 1997. Regional cell movement and tissue patterning in the zebrafish embryo revealed by fate mapping with caged fluorescein. Biochem Cell Biol. 75, 551-62.
Krauss, S., Concordet, J.P. and Ingham, P.W., 1993. A functionally conserved homolog of the Drosophila segment polarity gene hh is expressed in tissues with polarizing activity in zebrafish embryos. Cell. 75, 1431-44.
Lamb, T.M., Knecht, A.K., Smith, W.C., Stachel, S.E., Economides, A.N., Stahl, N., Yancopolous, G.D. and Harland, R.M., 1993. Neural induction by the secreted polypeptide noggin. Science. 262, 713-8.
Langenbacher, A. and Chen, J.N., 2008. Calcium signaling: a common thread in vertebrate left-right axis development. Dev Dyn. 237, 3491-6.
Larsen, C.W., Zeltser, L.M. and Lumsden, A., 2001. Boundary formation and compartition in the avian diencephalon. J Neurosci. 21, 4699-711.
Laufer, E., Nelson, C.E., Johnson, R.L., Morgan, B.A. and Tabin, C., 1994. Sonic Hedgehog and Fgf-4 Act through a Signaling Cascade and Feedback Loop to Integrate Growth and Patterning of the Developing Limb Bud. Cell. 79, 993-1003.
Launay, C., Fromentoux, V., Shi, D.L. and Boucaut, J.C., 1996. A truncated FGF receptor blocks neural induction by endogenous Xenopus inducers. Development. 122, 869-880.
Lechler, T. and Fuchs, E., 2007. Desmoplakin: an unexpected regulator of microtubule organization in the epidermis. J Cell Biol. 176, 147-54.
Levi, G., Mantero, S., Barbieri, O., Cantatore, D., Paleari, L., Beverdam, A., Genova, F., Robert, B. and Merlo, G.R., 2006. Msx1 and Dlx5 act independently in development of craniofacial skeleton, but converge on the regulation of Bmp signaling in palate formation. Mech Dev. 123, 3-16.
Levin, M., 1997. Left-right asymmetry in vertebrate embryogenesis. Bioessays. 19, 287-296.
Levin, M., 2003. Motor protein control of ion flux is an early step in embryonic left-right asymmetry. Bioessays. 25, 1002-10.

Levin, M., Johnson, R.L., Stern, C.D., Kuehn, M. and Tabin, C., 1995. A Molecular Pathway Determining Left-Right Asymmetry in Chick Embryogenesis. Cell. 82, 803-814.
Levin, M., Pagan, S., Roberts, D.J., Cooke, J., Kuehn, M.R. and Tabin, C.J., 1997. Left/right patterning signals and the independent regulation of different aspects of situs in the chick embryo. Dev Biol. 189, 57-67.
Lewandoski, M., Sun, X. and Martin, G.R., 2000. Fgf8 signalling from the AER is essential for normal limb development. Nat Genet. 26, 460-463.
Lewis, P.M., Dunn, M.P., McMahon, J.A., Logan, M., Martin, J.F., St-Jacques, B. and McMahon, A.P., 2001. Cholesterol modification of sonic hedgehog is required for long-range signaling activity and effective modulation of signaling by Ptc1. Cell. 105, 599-612.
Lewis, W.H., 1907. Transplantation of the lips of the blastopore in rana palustris. American Journal of Anatomy. 7, 137-143.
Leyns, L., Bouwmeester, T., Kim, S.H., Piccolo, S. and DeRobertis, E.M., 1997. Frzb-1 is a secreted antagonist of Wnt signaling expressed in the Spemann organizer. Cell. 88, 747-756.
Liem, K.F., Jr., Jessell, T.M. and Briscoe, J., 2000. Regulation of the neural patterning activity of sonic hedgehog by secreted BMP inhibitors expressed by notochord and somites. Development. 127, 4855-66.
Liem, K.F., Jr., Tremml, G. and Jessell, T.M., 1997. A role for the roof plate and its resident TGFbeta-related proteins in neuronal patterning in the dorsal spinal cord. Cell. 91, 127-38.
Liem, K.F., Jr., Tremml, G., Roelink, H. and Jessell, T.M., 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell. 82, 969-79.
Lin, W.J., Gary, J.D., Yang, M.C., Clarke, S. and Herschman, H.R., 1996. The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. J Biol Chem. 271, 15034-44.
Lindvall, J.M., Blomberg, K.E., Wennborg, A. and Smith, C.I., 2005. Differential expression and molecular characterisation of Lmo7, Myo1e, Sash1, and Mcoln2 genes in Btk-defective B-cells. Cell Immunol. 235, 46-55.
Linker, C., De Almeida, I., Papanayotou, C., Stower, M., Sabado, V., Ghorani, E., Streit, A., Mayor, R. and Stern, C.D., 2009. Cell communication with the neural plate is required for induction of neural markers by BMP inhibition: evidence for homeogenetic induction and implications for Xenopus animal cap and chick explant assays. Dev Biol. 327, 478-86.
Linker, C. and Stern, C.D., 2004. Neural induction requires BMP inhibition only as a late step, and involves signals other than FGF and Wnt antagonists. Development. 131, 5671-81.
Liu, A.M., Losos, K. and Joyner, A.L., 1999. FGF8 can activate Gbx2 and transform regions of the rostral mouse brain into a hindbrain. Development. 126, 4827-4838.
Liu, A.P., Majumdar, A., Schauerte, H.E., Haffter, P. and Drummond, I.A., 2000. Zebrafish wnt4b expression in the floor plate is altered in sonic hedgehog and gli-2 mutants. Mech Dev. 91, 409-413.
Long, S., Ahmad, N. and Rebagliati, M., 2003. The zebrafish nodal-related gene southpaw is required for visceral and diencephalic left-right asymmetry. Development. 130, 2303-2316.
Lopez-Martinez, A., Chang, D.T., Chiang, C., Porter, J.A., Ros, M.A., Simandl, B.K., Beachy, P.A. and Fallon, J.F., 1995. Limb-patterning activity and restricted posterior localization of the amino-terminal product of Sonic hedgehog cleavage. Curr Biol. 5, 791-6.

Lu, L.Y., Wood, J.L., Minter-Dykhouse, K., Ye, L., Saunders, T.L., Yu, X. and Chen, J., 2008a. Polo-like kinase 1 is essential for early embryonic development and tumor suppression. Mol Cell Biol. 28, 6870-6.

Lu, P., Yu, Y., Perdue, Y. and Werb, Z., 2008b. The apical ectodermal ridge is a timer for generating distal limb progenitors. Development. 135, 1395-405.

Lu, R., Alioua, A., Kumar, Y., Eghbali, M., Stefani, E. and Toro, L., 2006. MaxiK channel partners: physiological impact. J Physiol. 570, 65-72.

Ma, Q., Jones, D., Borghesani, P.R., Segal, R.A., Nagasawa, T., Kishimoto, T., Bronson, R.T. and Springer, T.A., 1998. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci U S A. 95, 9448-53.

Maecker, H.T., Todd, S.C. and Levy, S., 1997. The tetraspanin superfamily: molecular facilitators. FASEB J. 11, 428-42.

Maeda, K., Matsuhashi, S., Hori, K., Xin, Z., Mukai, T., Tabuchi, K., Egashira, M. and Niikawa, N., 1998. Cloning and characterization of a novel human gene, TM4SF6, encoding a protein belonging to the transmembrane 4 superfamily, and mapped to Xq22. Genomics. 52, 240-2.

Magleby, K.L., 2003. Gating mechanism of BK (Slo1) channels: so near, yet so far. J Gen Physiol. 121, 81-96.

Mains, R.E., Berard, C.A., Denault, J.B., Zhou, A., Johnson, R.C. and Leduc, R., 1997. PACE4: a subtilisin-like endoprotease with unique properties. Biochem J. 321 (Pt 3), 587-93.

Marazzi, G., Wang, Y. and Sassoon, D., 1997. Msx2 is a transcriptional regulator in the BMP4-mediated programmed cell death pathway. Dev Biol. 186, 127-38.

Mariani, F.V., Ahn, C.P. and Martin, G.R., 2008. Genetic evidence that FGFs have an instructive role in limb proximal-distal patterning. Nature. 453, 401-U56.

Marigo, V., Davey, R.A., Zuo, Y., Cunningham, J.M. and Tabin, C.J., 1996. Biochemical evidence that patched is the Hedgehog receptor. Nature. 384, 176-9.

Marin, F. and Puelles, L., 1994. Patterning of the embryonic avian midbrain after experimental inversions: a polarizing activity from the isthmus. Dev Biol. 163, 19-37.

Marin, O., Blanco, M.J. and Nieto, M.A., 2001. Differential expression of Eph receptors and ephrins correlates with the formation of topographic projections in primary and secondary visual circuits of the embryonic chick forebrain. Dev Biol. 234, 289-303.

Marti, E., Bumcrot, D.A., Takada, R. and McMahon, A.P., 1995. Requirement of 19K form of Sonic hedgehog for induction of distinct ventral cell types in CNS explants. Nature. 375, 322-5.

Martinez-Ferre, A., Navarro-Garberi, M., Bueno, C. and Martinez, S., 2013. Wnt Signal Specifies the Intrathalamic Limit and Its Organizer Properties by Regulating Shh Induction in the Alar Plate. Journal of Neuroscience. 33, 3967-3980.

Martinez-Morales, J.R., Marti, E., Frade, J.M. and Rodriguez-Tebar, A., 1995. Developmentally regulated vitronectin influences cell differentiation, neuron survival and process outgrowth in the developing chicken retina. Neuroscience. 68, 245-53.

Martinez, S., Marin, F., Nieto, M.A. and Puelles, L., 1995. Induction of Ectopic Engrailed Expression and Fate Change in Avian Rhombomeres - Intersegmental Boundaries as Barriers. Mech Dev. 51, 289-303.

Martinez, S., Wassef, M. and Alvarado-Mallart, R.M., 1991. Induction of a mesencephalic phenotype in the 2-day-old chick prosencephalon is preceded by the early expression of the homeobox gene en. Neuron. 6, 971-81.

Martinsen, B.J. and Bronner-Fraser, M., 1998. Neural crest specification regulated by the helix-loop-helix repressor Id2. Science. 281, 988-91.
Masai, I., Heisenberg, C.P., Barth, K.A., Macdonald, R., Adamek, S. and Wilson, S.W., 1997. floating head and masterblind regulate neuronal patterning in the roof of the forebrain. Neuron. 18, 43-57.
Masse, K., Bhamra, S., Allsop, G., Dale, N. and Jones, E.A., 2010. Ectophosphodiesterase/nucleotide phosphohydrolase (Enpp) nucleotidases: cloning, conservation and developmental restriction. Int J Dev Biol. 54, 181-93.
Matsuda, K., Miura, E., Miyazaki, T., Kakegawa, W., Emi, K., Narumi, S., Fukazawa, Y., Ito-Ishida, A., Kondo, T., Shigemoto, R., Watanabe, M. and Yuzaki, M., 2010. Cbln1 is a ligand for an orphan glutamate receptor delta2, a bidirectional synapse organizer. Science. 328, 363-8.
Matsunaga, E., Katahira, T. and Nakamura, H., 2002. Role of Lmx1b and Wnt1 in mesencephalon and metencephalon development. Development. 129, 5269-5277.
Mattes, B., Weber, S., Peres, J., Chen, Q., Davidson, G., Houart, C. and Scholpp, S., 2012. Wnt3 and Wnt3a are required for induction of the mid-diencephalic organizer in the caudal forebrain. Neural Dev. 7, 12.
McGrath, J., Somlo, S., Makova, S., Tian, X. and Brueckner, M., 2003. Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell. 114, 61-73.
McGrath, K.E., Koniski, A.D., Maltby, K.M., McGann, J.K. and Palis, J., 1999. Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4. Dev Biol. 213, 442-56.
Mcgrew, L.L., Otte, A.P. and Moon, R.T., 1992. Analysis of Xwnt-4 in Embryos of Xenopus-Laevis - a Wnt Family Member Expressed in the Brain and Floor Plate. Development. 115, 463-473.
McLennan, R. and Kulesa, P.M., 2007. In vivo analysis reveals a critical role for neuropilin-1 in cranial neural crest cell migration in chick. Dev Biol. 301, 227-39.
Mcmahon, A.P., Joyner, A.L., Bradley, A. and Mcmahon, J.A., 1992. The Midbrain Hindbrain Phenotype of Wnt-1--Wnt-1- Mice Results from Stepwise Deletion of Engrailed-Expressing Cells by 9.5 Days Postcoitum. Cell. 69, 581-595.
Meera, P., Wallner, M., Jiang, Z. and Toro, L., 1996. A calcium switch for the functional coupling between alpha (hslo) and beta subunits (KV,Ca beta) of maxi K channels. FEBS Lett. 382, 84-8.
Metcalf, D., Greenhalgh, C.J., Viney, E., Willson, T.A., Starr, R., Nicola, N.A., Hilton, D.J. and Alexander, W.S., 2000. Gigantism in mice lacking suppressor of cytokine signalling-2. Nature. 405, 1069-73.
Miao, H.Q., Soker, S., Feiner, L., Alonso, J.L., Raper, J.A. and Klagsbrun, M., 1999. Neuropilin-1 mediates collapsin-1/semaphorin III inhibition of endothelial cell motility: functional competition of collapsin-1 and vascular endothelial growth factor-165. J Cell Biol. 146, 233-42.
Migdal, M., Huppertz, B., Tessler, S., Comforti, A., Shibuya, M., Reich, R., Baumann, H. and Neufeld, G., 1998. Neuropilin-1 is a placenta growth factor-2 receptor. J Biol Chem. 273, 22272-8.
Millett, S., Campbell, K., Epstein, D.J., Losos, K., Harris, E. and Joyner, A.L., 1999. A role for Gbx2 in repression of Otx2 and positioning the mid/hindbrain organizer. Nature. 401, 161-164.
Missler, M., Hammer, R.E. and Sudhof, T.C., 1998. Neurexophilin binding to alpha-neurexins. A single LNS domain functions as an independently folding ligand-binding unit. J Biol Chem. 273, 34716-23.

Miura, E., Iijima, T., Yuzaki, M. and Watanabe, M., 2006. Distinct expression of Cbln family mRNAs in developing and adult mouse brains. Eur J Neurosci. 24, 750-60.
Mole, D.R., Blancher, C., Copley, R.R., Pollard, P.J., Gleadle, J.M., Ragoussis, J. and Ratcliffe, P.J., 2009. Genome-wide association of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha DNA binding with expression profiling of hypoxia-inducible transcripts. J Biol Chem. 284, 16767-75.
Moore-Scott, B.A., Opoka, R., Lin, S.C., Kordich, J.J. and Wells, J.M., 2007. Identification of molecular markers that are expressed in discrete anterior-posterior domains of the endoderm from the gastrula stage to mid-gestation. Dev Dyn. 236, 1997-2003.
Morton, M.J., Hutchinson, K., Mathieson, P.W., Witherden, I.R., Saleem, M.A. and Hunter, M., 2004. Human podocytes possess a stretch-sensitive, Ca2+-activated K+ channel: potential implications for the control of glomerular filtration. J Am Soc Nephrol. 15, 2981-7.
Mukhopadhyay, M., Teufel, A., Yamashita, T., Agulnick, A.D., Chen, L., Downs, K.M., Schindler, A., Grinberg, A., Huang, S.P., Dorward, D. and Westphal, H., 2003. Functional ablation of the mouse Ldb1 gene results in severe patterning defects during gastrulation. Development. 130, 495-505.
Muramatsu, T., Mizutani, Y., Ohmori, Y. and Okumura, J., 1997. Comparison of three nonviral transfection methods for foreign gene expression in early chicken embryos in ovo. Biochemical and Biophysical Research Communications. 230, 376-380.
Muto, A., Aoki, Y. and Watanabe, S., 2007. Mouse Rab11-FIP4 regulates proliferation and differentiation of retinal progenitors in a Rab11-independent manner. Dev Dyn. 236, 214-25.
Muto, A., Arai, K. and Watanabe, S., 2006. Rab11-FIP4 is predominantly expressed in neural tissues and involved in proliferation as well as in differentiation during zebrafish retinal development. Dev Biol. 292, 90-102.
Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Kikutani, H. and Kishimoto, T., 1996. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 382, 635-8.
Nagata, K., Suzuki, H., Niiya-Kato, A., Kinoshita, S., Taketani, S. and Araki, M., 2006. Neurensin-1 expression in the mouse retina during postnatal development and in the cultured retinal neurons. Brain Res. 1081, 65-71.
Nakahiro, T., Kurooka, H., Mori, K., Sano, K. and Yokota, Y., 2010. Identification of BMP-responsive elements in the mouse Id2 gene. Biochem Biophys Res Commun. 399, 416-21.
Nakamura, H. and Itasaki, N., 1992. Expression of En in the Prosencephalon Heterotopically Transplanted into the Mesencephalon. Development Growth & Differentiation. 34, 387-391.
Nakamura, H., Katahira, T., Sato, T., Watanabe, Y. and Funahashi, J., 2004. Gain- and loss-of-function in chick embryos by electroporation. Mech Dev. 121, 1137-1143.
Nakamura, H., Matsui, K.A., Takagi, S. and Fujisawa, H., 1991. Projection of the retinal ganglion cells to the tectum differentiated from the prosencephalon. Neurosci Res. 11, 189-97.
Nakamura, H., Nakano, K.E., Igawa, H.H., Takagi, S. and Fujisawa, H., 1986. Plasticity and rigidity of differentiation of brain vesicles studied in quail-chick chimeras. Cell Differ. 19, 187-93.
Nakamura, H., Watanabe, Y. and Funahashi, J., 2000. Misexpression of genes in brain vesicles by in ovo electroporation. Development Growth & Differentiation. 42, 199-201.

Negishi, M., Oinuma, I. and Katoh, H., 2005. Plexins: axon guidance and signal transduction. Cell Mol Life Sci. 62, 1363-71.
Nguyen, V.H., Schmid, B., Trout, J., Connors, S.A., Ekker, M. and Mullins, M.C., 1998. Ventral and lateral regions of the zebrafish gastrula, including the neural crest progenitors, are established by a bmp2b/swirl pathway of genes. Dev Biol. 199, 93-110.
Niederreither, K., Subbarayan, V., Dolle, P. and Chambon, P., 1999. Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. Nat Genet. 21, 444-448.
Niederreither, K., Vermot, J., Schuhbaur, B., Chambon, P. and Dolle, P., 2002. Embryonic retinoic acid synthesis is required for forelimb growth and anteroposterior patterning in the mouse. Development. 129, 3563-3574.
Niehrs, C., 2004. Regionally specific induction by the Spemann-Mangold organizer. Nat Rev Genet. 5, 425-34.
Niehrs, C., 2006. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene. 25, 7469-81.
Niehrs, C. and Pollet, N., 1999. Synexpression groups in eukaryotes. Nature. 402, 483-7.
Nieuwenhuys, R., 1999. The morphological pattern of the vertebrate brain. Eur J Morphol. 37, 81-4.
Nieuwkoop, P.D., 1992. The Formation of the Mesoderm in Urodelean Amphibians .6. The Self-Organizing Capacity of the Induced Meso-Endoderm. Rouxs Archives of Developmental Biology. 201, 18-29.
Nigg, E.A., 1998. Polo-like kinases: positive regulators of cell division from start to finish. Curr Opin Cell Biol. 10, 776-83.
Nishikawa, S., Ogawa, M., Nishikawa, S., Kunisada, T. and Kodama, H., 1988. B lymphopoiesis on stromal cell clone: stromal cell clones acting on different stages of B cell differentiation. Eur J Immunol. 18, 1767-71.
Nishimura, Y., Miyazawa, T., Ikeda, Y., Izumiya, Y., Nakamura, K., Cai, J.S., Sato, E., Kohmoto, M. and Mikami, T., 1998. Molecular cloning and sequencing of feline stromal cell-derived factor-1 alpha and beta. Eur J Immunogenet. 25, 303-5.
Niswander, L., Jeffrey, S., Martin, G.R. and Tickle, C., 1994. A positive feedback loop coordinates growth and patterning in the vertebrate limb. Nature. 371, 609-12.
Niswander, L., Tickle, C., Vogel, A., Booth, I. and Martin, G.R., 1993. Fgf-4 Replaces the Apical Ectodermal Ridge and Directs Outgrowth and Patterning of the Limb. Cell. 75, 579-587.
Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M. and Hirokawa, N., 1998. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell. 95, 829-37.
Okada, I., Hamanoue, H., Terada, K., Tohma, T., Megarbane, A., Chouery, E., Abou-Ghoch, J., Jalkh, N., Cogulu, O., Ozkinay, F., Horie, K., Takeda, J., Furuichi, T., Ikegawa, S., Nishiyama, K., Miyatake, S., Nishimura, A., Mizuguchi, T., Niikawa, N., Hirahara, F., Kaname, T., Yoshiura, K., Tsurusaki, Y., Doi, H., Miyake, N., Furukawa, T., Matsumoto, N. and Saitsu, H., 2011. SMOC1 is essential for ocular and limb development in humans and mice. Am J Hum Genet. 88, 30-41.
Okada, Y., Nonaka, S., Tanaka, Y., Saijoh, Y., Hamada, H. and Hirokawa, N., 1999. Abnormal nodal flow precedes situs inversus in iv and inv mice. Mol Cell. 4, 459-68.
Olsen, S.R. and Uhler, M.D., 1991. Isolation and characterization of cDNA clones for an inhibitor protein of cAMP-dependent protein kinase. J Biol Chem. 266, 11158-62.

Oppenheimer, J.M., 1936a. Structures developed in amphibians by implantation of living fish organizer. Proceedings of the Society for Experimental Biology and Medicine. 34, 461-463.
Oppenheimer, J.M., 1936b. Transplantation experiments on developing teleosts (Fundulus and perca). Journal of Experimental Zoology. 72, 409-437.
Orstavik, S., Natarajan, V., Tasken, K., Jahnsen, T. and Sandberg, M., 1997. Characterization of the human gene encoding the type I alpha and type I beta cGMP-dependent protein kinase (PRKG1). Genomics. 42, 311-8.
Oshimori, N., Ohsugi, M. and Yamamoto, T., 2006. The Plk1 target Kizuna stabilizes mitotic centrosomes to ensure spindle bipolarity. Nat Cell Biol. 8, 1095-101.
Ott, E.B., van den Akker, N.M., Sakalis, P.A., Gittenberger-de Groot, A.C., Te Velthuis, A.J. and Bagowski, C.P., 2008. The lim domain only protein 7 is important in zebrafish heart development. Dev Dyn. 237, 3940-52.
Panganiban, G. and Rubenstein, J.L., 2002. Developmental functions of the Distal-less/Dlx homeobox genes. Development. 129, 4371-86.
Panhuysen, M., Weisenhorn, D.M.V., Blanquet, V., Brodski, C., Heinzmann, U., Beisker, W. and Wurst, W., 2004. Effects of Wnt1 signaling on proliferation in the developing mid-/hindbrain region. Molecular and Cellular Neuroscience. 26, 101-111.
Park, S., Lee, Y.J., Lee, H.J., Seki, T., Hong, K.H., Park, J., Beppu, H., Lim, I.K., Yoon, J.W., Li, E., Kim, S.J. and Oh, S.P., 2004. B-cell translocation gene 2 (Btg2) regulates vertebral patterning by modulating bone morphogenetic protein/smad signaling. Mol Cell Biol. 24, 10256-62.
Parrington, J., Swann, K., Shevchenko, V.I., Sesay, A.K. and Lai, F.A., 1996. Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. Nature. 379, 364-8.
Pasterkamp, R.J., Kolk, S.M., Hellemons, A.J. and Kolodkin, A.L., 2007. Expression patterns of semaphorin7A and plexinC1 during rat neural development suggest roles in axon guidance and neuronal migration. BMC Dev Biol. 7, 98.
Patten, I., Kulesa, P., Shen, M.M., Fraser, S. and Placzek, M., 2003. Distinct modes of floor plate induction in the chick embryo. Development. 130, 4809-4821.
Perala, N.M., Immonen, T. and Sariola, H., 2005. The expression of plexins during mouse embryogenesis. Gene Expr Patterns. 5, 355-62.
Perron, J.C. and Bixby, J.L., 1999. Tetraspanins expressed in the embryonic chick nervous system. FEBS Lett. 461, 86-90.
Peter, I.S. and Davidson, E.H., 2009. Genomic control of patterning. Int J Dev Biol. 53, 707-16.
Petrenko, A.G., Ullrich, B., Missler, M., Krasnoperov, V., Rosahl, T.W. and Sudhof, T.C., 1996. Structure and evolution of neurexophilin. J Neurosci. 16, 4360-9.
Piccolo, S., Agius, E., Leyns, L., Bhattacharyya, S., Grunz, H., Bouwmeester, T. and De Robertis, E.M., 1999. The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. Nature. 397, 707-710.
Piccolo, S., Sasai, Y., Lu, B. and DeRobertis, E.M., 1996. Dorsoventral patterning in xenopus: Inhibition of ventral signals by direct binding of Chordin to BMP-4. Cell. 86, 589-598.
Pispa, J., Mikkola, M.L., Mustonen, T. and Thesleff, I., 2003. Ectodysplasin, Edar and TNFRSF19 are expressed in complementary and overlapping patterns during mouse embryogenesis. Gene Expr Patterns. 3, 675-9.
Pitulescu, M.E. and Adams, R.H., 2010. Eph/ephrin molecules--a hub for signaling and endocytosis. Genes Dev. 24, 2480-92.
Placzek, M., Jessell, T.M. and Dodd, J., 1993. Induction of floor plate differentiation by contact-dependent, homeogenetic signals. Development. 117, 205-18.

Placzek, M., Tessierlavigne, M., Yamada, T., Jessell, T. and Dodd, J., 1990. Mesodermal Control of Neural Cell Identity - Floor Plate Induction by the Notochord. Science. 250, 985-988.
Placzek, M., Yamada, T., Tessier-Lavigne, M., Jessell, T. and Dodd, J., 1991. Control of dorsoventral pattern in vertebrate neural development: induction and polarizing properties of the floor plate. Development. Suppl 2, 105-22.
Plouhinec, J.L., Granier, C., Le Mentec, C., Lawson, K.A., Saberan-Djoneidi, D., Aghion, J., Shi, D.L., Collignon, J. and Mazan, S., 2004. Identification of the mammalian Not gene via a phylogenomic approach. Gene Expr Patterns. 5, 11-22.
Polizzotto, M.N., Bartlett, P.F. and Turnley, A.M., 2000. Expression of "suppressor of cytokine signalling" (SOCS) genes in the developing and adult mouse nervous system. J Comp Neurol. 423, 348-58.
Pons, S. and Marti, E., 2000. Sonic hedgehog synergizes with the extracellular matrix protein vitronectin to induce spinal motor neuron differentiation. Development. 127, 333-42.
Pouyssegur, J., Dayan, F. and Mazure, N.M., 2006. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature. 441, 437-43.
Preissner, K.T., 1991. Structure and biological role of vitronectin. Annu Rev Cell Biol. 7, 275-310.
Prevot, D., Morel, A.P., Voeltzel, T., Rostan, M.C., Rimokh, R., Magaud, J.P. and Corbo, L., 2001. Relationships of the antiproliferative proteins BTG1 and BTG2 with CAF1, the human homolog of a component of the yeast CCR4 transcriptional complex: involvement in estrogen receptor alpha signaling pathway. J Biol Chem. 276, 9640-8.
Prevot, D., Voeltzel, T., Birot, A.M., Morel, A.P., Rostan, M.C., Magaud, J.P. and Corbo, L., 2000. The leukemia-associated protein Btg1 and the p53-regulated protein Btg2 interact with the homeoprotein Hoxb9 and enhance its transcriptional activation. J Biol Chem. 275, 147-53.
Prince, J.E., Brignall, A.C., Cutforth, T., Shen, K. and Cloutier, J.F., 2013. Kirrel3 is required for the coalescence of vomeronasal sensory neuron axons into glomeruli and for male-male aggression. Development. 140, 2398-408.
Psychoyos, D. and Stern, C.D., 1996. Restoration of the organizer after radical ablation of Hensen's node and the anterior primitive streak in the chick embryo. Development. 122, 3263-73.
Puelles, L., Amat, J.A. and Martinez-de-la-Torre, M., 1987. Segment-related, mosaic neurogenetic pattern in the forebrain and mesencephalon of early chick embryos: I. Topography of AChE-positive neuroblasts up to stage HH18. J Comp Neurol. 266, 247-68.
Qian, Y., Chao, D.S., Santillano, D.R., Cornwell, T.L., Nairn, A.C., Greengard, P., Lincoln, T.M. and Bredt, D.S., 1996. cGMP-dependent protein kinase in dorsal root ganglion: relationship with nitric oxide synthase and nociceptive neurons. J Neurosci. 16, 3130-8.
Quina, L.A., Wang, S., Ng, L. and Turner, E.E., 2009. Brn3a and Nurr1 mediate a gene regulatory pathway for habenula development. J Neurosci. 29, 14309-22.
Quinlan, R., Graf, M., Mason, I., Lumsden, A. and Kiecker, C., 2009. Complex and dynamic patterns of Wnt pathway gene expression in the developing chick forebrain. Neural Dev. 4, 35.
Rainger, J., van Beusekom, E., Ramsay, J.K., McKie, L., Al-Gazali, L., Pallotta, R., Saponari, A., Branney, P., Fisher, M., Morrison, H., Bicknell, L., Gautier, P., Perry, P., Sokhi, K., Sexton, D., Bardakjian, T.M., Schneider, A.S., Elcioglu, N., Ozkinay, F., Koenig, R., Megarbane, A., Semerci, C.N., Khan, A., Zafar, S., Hennekam, R., Sousa, S.B.,

Ramos, L., Garavelli, L., Furga, A.S., Wischmeijer, A., Jackson, I.J., Gillessen-Kaesbach, G., Brunner, H.G., Wieczorek, D., van Bokhoven, H. and Fitzpatrick, D.R., 2011. Loss of the BMP antagonist, SMOC-1, causes Ophthalmo-acromelic (Waardenburg Anophthalmia) syndrome in humans and mice. PLoS Genet. 7, e1002114.

Ranson, M., Tickle, C., Mahon, K.A. and Mackem, S., 1995. Gnot1, a member of a new homeobox gene subfamily, is expressed in a dynamic, region-specific domain along the proximodistal axis of the developing limb. Mech Dev. 51, 17-30.

Rash, B.G. and Grove, E.A., 2007. Patterning the dorsal telencephalon: A role for sonic hedgehog? Journal of Neuroscience. 27, 11595-11603.

Read, L.R., Cumberbatch, J.A., Buhr, M.M., Bendall, A.J. and Sharif, S., 2005. Cloning and characterization of chicken stromal cell derived factor-1. Dev Comp Immunol. 29, 143-52.

Rehimi, R., Khalida, N., Yusuf, F., Dai, F., Morosan-Puopolo, G. and Brand-Saberi, B., 2008. Stromal-derived factor-1 (SDF-1) expression during early chick development. Int J Dev Biol. 52, 87-92.

Reiner, A., Yang, M., Cagle, M.C. and Honig, M.G., 2011. Localization of cerebellin-2 in late embryonic chicken brain: implications for a role in synapse formation and for brain evolution. J Comp Neurol. 519, 2225-51.

Renaud, J., Kerjan, G., Sumita, I., Zagar, Y., Georget, V., Kim, D., Fouquet, C., Suda, K., Sanbo, M., Suto, F., Ackerman, S.L., Mitchell, K.J., Fujisawa, H. and Chedotal, A., 2008. Plexin-A2 and its ligand, Sema6A, control nucleus-centrosome coupling in migrating granule cells. Nat Neurosci. 11, 440-9.

Riccio, O., Hurni, N., Murthy, S., Vutskits, L., Hein, L. and Dayer, A., 2012. Alpha2-adrenergic receptor activation regulates cortical interneuron migration. Eur J Neurosci. 36, 2879-87.

Riddle, R.D., Johnson, R.L., Laufer, E. and Tabin, C., 1993a. Sonic-Hedgehog Mediates the Polarizing Activity of the Zpa. Cell. 75, 1401-1416.

Riddle, R.D., Johnson, R.L., Laufer, E. and Tabin, C., 1993b. Sonic hedgehog mediates the polarizing activity of the ZPA. Cell. 75, 1401-16.

Ringwald, M., Baldock, R., Bard, J., Kaufman, M., Eppig, J.T., Richardson, J.E., Nadeau, J.H. and Davidson, D., 1994. A database for mouse development. Science. 265, 2033-4.

Rivera-Perez, J.A., Mallo, M., Gendron-Maguire, M., Gridley, T. and Behringer, R.R., 1995. Goosecoid is not an essential component of the mouse gastrula organizer but is required for craniofacial and rib development. Development. 121, 3005-12.

Robert, B., Sassoon, D., Jacq, B., Gehring, W. and Buckingham, M., 1989. Hox-7, a mouse homeobox gene with a novel pattern of expression during embryogenesis. EMBO J. 8, 91-100.

Robertson, C.P., Gibbs, S.M. and Roelink, H., 2001. cGMP enhances the sonic hedgehog response in neural plate cells. Dev Biol. 238, 157-67.

Robledo, R.F., Rajan, L., Li, X. and Lufkin, T., 2002. The Dlx5 and Dlx6 homeobox genes are essential for craniofacial, axial, and appendicular skeletal development. Genes Dev. 16, 1089-101.

Roelink, H., Augsburger, A., Heemskerk, J., Korzh, V., Norlin, S., Altaba, A.R.I., Tanabe, Y., Placzek, M., Edlund, T., Jessell, T.M. and Dodd, J., 1994. Floor Plate and Motor-Neuron Induction by Vhh-1, a Vertebrate Homolog of Hedgehog Expressed by the Notochord. Cell. 76, 761-775.

Roney, K., Holl, E. and Ting, J., 2013. Immune plexins and semaphorins: old proteins, new immune functions. Protein Cell. 4, 17-26.

Rong, Y., Wei, P., Parris, J., Guo, H., Pattarini, R., Correia, K., Li, L., Kusnoor, S.V., Deutch, A.Y. and Morgan, J.I., 2012. Comparison of Cbln1 and Cbln2 functions using transgenic and knockout mice. J Neurochem. 120, 528-40.

Rosenblatt, K.P., Sun, Z.P., Heller, S. and Hudspeth, A.J., 1997. Distribution of Ca2+-activated K+ channel isoforms along the tonotopic gradient of the chicken's cochlea. Neuron. 19, 1061-75.

Rouault, J.P., Falette, N., Guehenneux, F., Guillot, C., Rimokh, R., Wang, Q., Berthet, C., Moyret-Lalle, C., Savatier, P., Pain, B., Shaw, P., Berger, R., Samarut, J., Magaud, J.P., Ozturk, M., Samarut, C. and Puisieux, A., 1996. Identification of BTG2, an antiproliferative p53-dependent component of the DNA damage cellular response pathway. Nat Genet. 14, 482-6.

Rubenstein, J.L., Martinez, S., Shimamura, K. and Puelles, L., 1994. The embryonic vertebrate forebrain: the prosomeric model. Science. 266, 578-80.

Ruiz i Altaba, A., 1997. Catching a Gli-mpse of Hedgehog. Cell. 90, 193-6.

Ruiz i Altaba, A., Placzek, M., Baldassare, M., Dodd, J. and Jessell, T.M., 1995. Early stages of notochord and floor plate development in the chick embryo defined by normal and induced expression of HNF-3 beta. Dev Biol. 170, 299-313.

Salkoff, L., Butler, A., Ferreira, G., Santi, C. and Wei, A., 2006. High-conductance potassium channels of the SLO family. Nat Rev Neurosci. 7, 921-31.

Salvi, E., Buffa, R. and Renda, T.G., 1996. Ontogeny, distribution and amine/peptide content of chromogranin A- and B-immunoreactive endocrine cells in the small and large intestine of the chicken. Anat Embryol (Berl). 194, 89-98.

Sanchez-Garcia, I. and Rabbitts, T.H., 1994. The LIM domain: a new structural motif found in zinc-finger-like proteins. Trends Genet. 10, 315-20.

Sanders, T.A., Llagostera, E. and Barna, M., 2013. Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. Nature. 497, 628-32.

Sasai, Y., Lu, B., Piccolo, S. and DeRobertis, E.M., 1996. Endoderm induction by the organizer-secreted factors chordin and noggin in Xenopus animal caps. Embo Journal. 15, 4547-4555.

Sasaki, H., Hui, C., Nakafuku, M. and Kondoh, H., 1997. A binding site for Gli proteins is essential for HNF-3beta floor plate enhancer activity in transgenics and can respond to Shh in vitro. Development. 124, 1313-22.

Sato, T., Araki, I. and Nakamura, H., 2001. Inductive signal and tissue responsiveness defining the tectum and the cerebellum. Development. 128, 2461-2469.

Sato, T. and Joyner, A.L., 2009. The duration of Fgf8 isthmic organizer expression is key to patterning different tectal-isthmo-cerebellum structures. Development. 136, 3617-26.

Sato, T. and Nakamura, H., 2004. The Fgf8 signal causes cerebellar differentiation by activating the Ras-ERK signaling pathway. Development. 131, 4275-4285.

Satoh, J., Obayashi, S., Tabunoki, H., Wakana, T. and Kim, S.U., 2010. Stable expression of neurogenin 1 induces LGR5, a novel stem cell marker, in an immortalized human neural stem cell line HB1.F3. Cell Mol Neurobiol. 30, 415-26.

Saunders, J.W., Jr., 1948. The proximo-distal sequence of origin of the parts of the chick wing and the role of the ectoderm. J Exp Zool. 108, 363-403.

Schmidt, J., Francois, V., Bier, E. and Kimelman, D., 1995. Drosophila short gastrulation induces an ectopic axis in Xenopus: evidence for conserved mechanisms of dorsal-ventral patterning. Development. 121, 4319-28.

Scholpp, S., Foucher, I., Staudt, N., Peukert, D., Lumsden, A. and Houart, C., 2007. Otx1l, Otx2 and Irx1b establish and position the ZLI in the diencephalon. Development. 134, 3167-76.

Scholpp, S. and Lumsden, A., 2010. Building a bridal chamber: development of the thalamus. Trends Neurosci. 33, 373-80.
Scholpp, S., Wolf, O., Brand, M. and Lumsden, A., 2006. Hedgehog signalling from the zona limitans intrathalamica orchestrates patterning of the zebrafish diencephalon. Development. 133, 855-864.
Scott, H.J., Stebbing, M.J., Walters, C.E., McLenachan, S., Ransome, M.I., Nichols, N.R. and Turnley, A.M., 2006. Differential effects of SOCS2 on neuronal differentiation and morphology. Brain Res. 1067, 138-45.
Seidah, N.G., Mayer, G., Zaid, A., Rousselet, E., Nassoury, N., Poirier, S., Essalmani, R. and Prat, A., 2008. The activation and physiological functions of the proprotein convertases. Int J Biochem Cell Biol. 40, 1111-25.
Seiffert, D., Iruela-Arispe, M.L., Sage, E.H. and Loskutoff, D.J., 1995. Distribution of vitronectin mRNA during murine development. Dev Dyn. 203, 71-9.
Selleck, M.A. and Stern, C.D., 1991. Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. Development. 112, 615-26.
Semenova, E., Wang, X., Jablonski, M.M., Levorse, J. and Tilghman, S.M., 2003. An engineered 800 kilobase deletion of Uchl3 and Lmo7 on mouse chromosome 14 causes defects in viability, postnatal growth and degeneration of muscle and retina. Hum Mol Genet. 12, 1301-12.
Sgaier, S.K., Lao, Z.M., Villanueva, M.P., Berenshteyn, F., Stephen, D., Turnbull, R.K. and Joyner, A.L., 2007. Genetic subdivision of the tectum and cerebellum into functionally related regions based on differential sensitivity to engrailed proteins. Development. 134, 2325-2335.
Shanmugalingam, S., Houart, C., Picker, A., Reifers, F., Macdonald, R., Barth, A., Griffin, K., Brand, M. and Wilson, S.W., 2000. Ace/Fgf8 is required for forebrain commissure formation and patterning of the telencephalon. Development. 127, 2549-2561.
Sharpe, J., 2003. Optical projection tomography as a new tool for studying embryo anatomy. J Anat. 202, 175-81.
Shevchenko, V., Hogben, M., Ekong, R., Parrington, J. and Lai, F.A., 1998. The human glucosamine-6-phosphate deaminase gene: cDNA cloning and expression, genomic organization and chromosomal localization. Gene. 216, 31-8.
Shih, J. and Fraser, S.E., 1996. Characterizing the zebrafish organizer: Microsurgical analysis at the early-shield stage. Development. 122, 1313-1322.
Shimamura, K. and Rubenstein, J.L., 1997a. Inductive interactions direct early regionalization of the mouse forebrain. Development. 124, 2709-18.
Shimamura, K. and Rubenstein, J.L.R., 1997b. Inductive interactions direct early regionalization of the mouse forebrain. Development. 124, 2709-2718.
Shimeld, S.M., McKay, I.J. and Sharpe, P.T., 1996. The murine homeobox gene Msx-3 shows highly restricted expression in the developing neural tube. Mech Dev. 55, 201-10.
Shinya, M., Koshida, S., Sawada, A., Kuroiwa, A. and Takeda, H., 2001. Fgf signalling through MAPK cascade is required for development of the subpallial telencephalon in zebrafish embryos. Development. 128, 4153-4164.
Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinohara, T. and Honjo, T., 1995. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. Genomics. 28, 495-500.
Slack, J.M., 1987. Embryology: we have a morphogen! Nature. 327, 553-4.
Snelson, C.D., Burkart, J.T. and Gamse, J.T., 2008. Formation of the asymmetric pineal complex in zebrafish requires two independently acting transcription factors. Dev Dyn. 237, 3538-44.

Soker, S., Takashima, S., Miao, H.Q., Neufeld, G. and Klagsbrun, M., 1998. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell. 92, 735-45.

Spemann, H., 1918. Über die Determination der ersten Organanlagen des Amphibienembryo I–VI. Archiv für Entwicklungsmechanik der Organismen 43, 448-555.

Spemann, H., 1924a. Vererbung und Entwicklungsmechanik. Naturwissenschaften 12, 65-79.

Spemann, H. and Mangold, H., 2001. Induction of embryonic primordia by implantation of organizers from a different species. 1923. Int J Dev Biol. 45, 13-38.

Spemann, H.M., H. Über 1924b. induktion von Embryonalagen durch Implantation Artfremder Organisatoren. . Roux' Arch. Entw. Mech. 100, 599–638.

Starr, R., Willson, T.A., Viney, E.M., Murray, L.J., Rayner, J.R., Jenkins, B.J., Gonda, T.J., Alexander, W.S., Metcalf, D., Nicola, N.A. and Hilton, D.J., 1997. A family of cytokine-inducible inhibitors of signalling. Nature. 387, 917-21.

Staudt, N. and Houart, C., 2007. The prethalamus is established during gastrulation and influences diencephalic regionalization. PLoS Biol. 5, e69.

Stein, S. and Kessel, M., 1995. A homeobox gene involved in node, notochord and neural plate formation of chick embryos. Mech Dev. 49, 37-48.

Stein, S., Niss, K. and Kessel, M., 1996. Differential activation of the clustered homeobox genes CNOT2 and CNOT1 during notogenesis in the chick. Dev Biol. 180, 519-33.

Stern, C.D., 2005. Neural induction: old problem, new findings, yet more questions. Development. 132, 2007-21.

Stern, C.D., Yu, R.T., Kakizuka, A., Kintner, C.R., Mathews, L.S., Vale, W.W., Evans, R.M. and Umesono, K., 1995. Activin and its receptors during gastrulation and the later phases of mesoderm development in the chick embryo. Dev Biol. 172, 192-205.

Stewart, L.C. and Klinman, J.P., 1988. Dopamine beta-hydroxylase of adrenal chromaffin granules: structure and function. Annu Rev Biochem. 57, 551-92.

Stock, D.W., Ellies, D.L., Zhao, Z., Ekker, M., Ruddle, F.H. and Weiss, K.M., 1996. The evolution of the vertebrate Dlx gene family. Proc Natl Acad Sci U S A. 93, 10858-63.

Storey, K.G., Crossley, J.M., De Robertis, E.M., Norris, W.E. and Stern, C.D., 1992. Neural induction and regionalisation in the chick embryo. Development. 114, 729-41.

Storey, K.G., Selleck, M.A. and Stern, C.D., 1995. Neural induction and regionalisation by different subpopulations of cells in Hensen's node. Development. 121, 417-28.

Streit, A., 2002. Extensive cell movements accompany formation of the otic placode. Dev Biol. 249, 237-54.

Streit, A., Berliner, A.J., Papanayotou, C., Sirulnik, A. and Stern, C.D., 2000. Initiation of neural induction by FGF signalling before gastrulation. Nature. 406, 74-8.

Streit, A., Lee, K.J., Wool, I., Roberts, C., Jessell, T.M. and Stern, C.D., 1998. Chordin regulates primitive streak development and the stability of induced neural cells, but is not sufficient for neural induction in the chick embryo. Development. 125, 507-519.

Streit, A.C. and Stern, C.D., 1997. Competence for neural induction: HGF/SF, HGF1/MSP and the c-Met receptor. Ciba Found Symp. 212, 155-65; discussion 165-8.

Supp, D.M., Witte, D.P., Potter, S.S. and Brueckner, M., 1997. Mutation of an axonemal dynein affects left-right asymmetry in inversus viscerum mice. Nature. 389, 963-6.

Susa, T., Sato, T., Ono, T., Kato, T. and Kato, Y., 2006. Cofactor CLIM2 promotes the repressive action of LIM homeodomain transcription factor Lhx2 in the expression of porcine pituitary glycoprotein hormone alpha subunit gene. Biochim Biophys Acta. 1759, 403-9.

Sutherland, R.J., 1982. The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. Neurosci Biobehav Rev. 6, 1-13.
Suzuki-Hirano, A., Harada, H., Sato, T. and Nakamura, H., 2010. Activation of Ras-ERK pathway by Fgf8 and its downregulation by Sprouty2 for the isthmus organizing activity. Dev Biol. 337, 284-93.
Suzuki, H., Tohyama, K., Nagata, K., Taketani, S. and Araki, M., 2007. Regulatory expression of Neurensin-1 in the spinal motor neurons after mouse sciatic nerve injury. Neurosci Lett. 421, 152-7.
Tabin, C.J., 2006. The key to left-right asymmetry. Cell. 127, 27-32.
Tachibana, K., Hirota, S., Iizasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., Kitamura, Y., Matsushima, K., Yoshida, N., Nishikawa, S., Kishimoto, T. and Nagasawa, T., 1998. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature. 393, 591-4.
Takagi, S., Kasuya, Y., Shimizu, M., Matsuura, T., Tsuboi, M., Kawakami, A. and Fujisawa, H., 1995. Expression of a cell adhesion molecule, neuropilin, in the developing chick nervous system. Dev Biol. 170, 207-22.
Talbot, W.S., Trevarrow, B., Halpern, M.E., Melby, A.E., Farr, G., Postlethwait, J.H., Jowett, T., Kimmel, C.B. and Kimelman, D., 1995. A homeobox gene essential for zebrafish notochord development. Nature. 378, 150-7.
Tamagnone, L., Artigiani, S., Chen, H., He, Z., Ming, G.I., Song, H., Chedotal, A., Winberg, M.L., Goodman, C.S., Poo, M., Tessier-Lavigne, M. and Comoglio, P.M., 1999. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell. 99, 71-80.
Tamplin, O.J., Kinzel, D., Cox, B.J., Bell, C.E., Rossant, J. and Lickert, H., 2008. Microarray analysis of Foxa2 mutant mouse embryos reveals novel gene expression and inductive roles for the gastrula organizer and its derivatives. BMC Genomics. 9, 511.
Tanabe, Y. and Jessell, T.M., 1996. Diversity and pattern in the developing spinal cord. Science. 274, 1115-23.
Tang, H., Ross, A. and Capel, B., 2008. Expression and functional analysis of Gm114, a putative mammalian ortholog of Drosophila bam. Dev Biol. 318, 73-81.
Tatemoto, K., Efendic, S., Mutt, V., Makk, G., Feistner, G.J. and Barchas, J.D., 1986. Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. Nature. 324, 476-8.
Thaler, J.P., Lee, S.K., Jurata, L.W., Gill, G.N. and Pfaff, S.L., 2002. LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. Cell. 110, 237-49.
Thaller, C. and Eichele, G., 1987. Identification and spatial distribution of retinoids in the developing chick limb bud. Nature. 327, 625-8.
Thomas, J.T., Canelos, P., Luyten, F.P. and Moos, M., Jr., 2009. Xenopus SMOC-1 Inhibits bone morphogenetic protein signaling downstream of receptor binding and is essential for postgastrulation development in Xenopus. J Biol Chem. 284, 18994-9005.
Tian, F., Fessenden, J.D. and Schacht, J., 1999. Cyclic GMP-dependent protein kinase-I in the guinea pig cochlea. Hear Res. 131, 63-70.
Tickle, C., Alberts, B., Wolpert, L. and Lee, J., 1982. Local application of retinoic acid to the limb bond mimics the action of the polarizing region. Nature. 296, 564-6.
Tiveron, M.C. and Cremer, H., 2008. CXCL12/CXCR4 signalling in neuronal cell migration. Curr Opin Neurobiol. 18, 237-44.
Todd, S.C., Doctor, V.S. and Levy, S., 1998. Sequences and expression of six new members of the tetraspanin/TM4SF family. Biochim Biophys Acta. 1399, 101-4.

Tomancak, P., Beaton, A., Weiszmann, R., Kwan, E., Shu, S., Lewis, S.E., Richards, S., Ashburner, M., Hartenstein, V., Celniker, S.E. and Rubin, G.M., 2002. Systematic determination of patterns of gene expression during Drosophila embryogenesis. Genome Biol. 3, RESEARCH0088.

Tonegawa, A., Funayama, N., Ueno, N. and Takahashi, Y., 1997. Mesodermal subdivision along the mediolateral axis in chicken controlled by different concentrations of BMP-4. Development. 124, 1975-84.

Tran, Y.H., Xu, Z., Kato, A., Mistry, A.C., Goya, Y., Taira, M., Brandt, S.J. and Hirose, S., 2006. Spliced isoforms of LIM-domain-binding protein (CLIM/NLI/Ldb) lacking the LIM-interaction domain. J Biochem. 140, 105-19.

Turnley, A.M., Faux, C.H., Rietze, R.L., Coonan, J.R. and Bartlett, P.F., 2002. Suppressor of cytokine signaling 2 regulates neuronal differentiation by inhibiting growth hormone signaling. Nat Neurosci. 5, 1155-62.

van de Weerdt, B.C. and Medema, R.H., 2006. Polo-like kinases: a team in control of the division. Cell Cycle. 5, 853-64.

Van Patten, S.M., Donaldson, L.F., McGuinness, M.P., Kumar, P., Alizadeh, A., Griswold, M.D. and Walsh, D.A., 1997. Specific testicular cellular localization and hormonal regulation of the PKIalpha and PKIbeta isoforms of the inhibitor protein of the cAMP-dependent protein kinase. J Biol Chem. 272, 20021-9.

van Straaten, H.W. and Hekking, J.W., 1991. Development of floor plate, neurons and axonal outgrowth pattern in the early spinal cord of the notochord-deficient chick embryo. Anat Embryol (Berl). 184, 55-63.

Vanhaesebroeck, B., Welham, M.J., Kotani, K., Stein, R., Warne, P.H., Zvelebil, M.J., Higashi, K., Volinia, S., Downward, J. and Waterfield, M.D., 1997. P110delta, a novel phosphoinositide 3-kinase in leukocytes. Proc Natl Acad Sci U S A. 94, 4330-5.

Vannahme, C., Smyth, N., Miosge, N., Gosling, S., Frie, C., Paulsson, M., Maurer, P. and Hartmann, U., 2002. Characterization of SMOC-1, a novel modular calcium-binding protein in basement membranes. J Biol Chem. 277, 37977-86.

Vanstraaten, H.W.M., Hekking, J.W.M., Thors, F., Wiertzhoessels, E.L.M.J. and Drukker, J., 1985. Induction of an Additional Floor Plate in the Neural-Tube. Acta Morphologica Neerlando-Scandinavica. 23, 91-97.

Varjosalo, M. and Taipale, J., 2008. Hedgehog: functions and mechanisms. Genes Dev. 22, 2454-72.

Vieira, C., Garda, A.L., Shimamura, K. and Martinez, S., 2005. Thalamic development induced by Shh in the chick embryo. Dev Biol. 284, 351-63.

Vieira, C. and Martinez, S., 2006. Sonic hedgehog from the basal plate and the zona limitans intrathalamica exhibits differential activity on diencephalic molecular regionalization and nuclear structure. Neuroscience. 143, 129-140.

Vieux-Rochas, M., Bouhali, K., Mantero, S., Garaffo, G., Provero, P., Astigiano, S., Barbieri, O., Caratozzolo, M.F., Tullo, A., Guerrini, L., Lallemand, Y., Robert, B., Levi, G. and Merlo, G.R., 2013. BMP-mediated functional cooperation between Dlx5;Dlx6 and Msx1;Msx2 during mammalian limb development. PLoS One. 8, e51700.

Villalon, A., Sepulveda, M., Guerrero, N., Meynard, M.M., Palma, K. and Concha, M.L., 2012. Evolutionary plasticity of habenular asymmetry with a conserved efferent connectivity pattern. PLoS One. 7, e35329.

Volker, L.A., Petry, M., Abdelsabour-Khalaf, M., Schweizer, H., Yusuf, F., Busch, T., Schermer, B., Benzing, T., Brand-Saberi, B., Kretz, O., Hohne, M. and Kispert, A., 2012. Comparative analysis of Neph gene expression in mouse and chicken development. Histochem Cell Biol. 137, 355-66.

von Dassow, G., Schmidt, J.E. and Kimelman, D., 1993. Induction of the Xenopus organizer: expression and regulation of Xnot, a novel FGF and activin-regulated homeo box gene. Genes Dev. 7, 355-66.
Vue, T.Y., Bluske, K., Alishahi, A., Yang, L.L., Koyano-Nakagawa, N., Novitch, B. and Nakagawa, Y., 2009. Sonic hedgehog signaling controls thalamic progenitor identity and nuclei specification in mice. J Neurosci. 29, 4484-97.
Wada, C. and Ohtani, H., 1991. Molecular cloning of rat cerebellin-like protein cDNA which encodes a novel membrane-associated glycoprotein. Brain Res Mol Brain Res. 9, 71-7.
Waddington, C.H., 1932a. Experiments on the development of chick and duck embryos, cultivated in vitro. Philosophical Transactions of the Royal Society of London Series B-Containing Papers of a Biological Character. 221, 179-230.
Waddington, C.H., 1932b. Experiments on the Development of Chick and Duck Embryos, Cultivated in vitro. Philosophical Transactions of the Royal Society of London. Series B, Containing Papers of a Biological Character 221, 179–230.
Waddington, C.H., 1933. Induction by the primitive streak and its derivatives in the chick. Journal of Experimental Biology. 10, 38-U4.
Waddington, C.H., 1934. Experiments on embryonic induction Part I. The competence of the extra-embryonic ectoderm in the chick Part II. Experiments on coagulated organisers in the chick Part III. A note on inductions by chick primitive streak transplanted to the rabbit embryo. Journal of Experimental Biology. 11, 211-227.
Waddington, C.H., 1936. Organizers in mammalian development. Nature. 138, 125-125.
Waddington, C.H., 1937. Experiments on determination in the rabbit embryo. Arch. Biol. 48, 273-290.
Wakamatsu, Y., 2009. Overlapped and differential expression of cAMP-dependent kinase-inhibitor isoforms during avian organogenesis period. Dev Growth Differ. 51, 707-14.
Wang, R.X. and Limbird, L.E., 1997. Distribution of mRNA encoding three alpha 2-adrenergic receptor subtypes in the developing mouse embryo suggests a role for the alpha 2A subtype in apoptosis. Mol Pharmacol. 52, 1071-80.
Watabe, T., Kim, S., Candia, A., Rothbacher, U., Hashimoto, C., Inoue, K. and Cho, K.W.Y., 1995. Molecular mechanisms of Spemann's organizer formation: Conserved growth factor synergy between Xenopus and mouse. Genes Dev. 9, 3038-3050.
Weigmann, A., Corbeil, D., Hellwig, A. and Huttner, W.B., 1997. Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. Proc Natl Acad Sci U S A. 94, 12425-30.
Weiler, S., Rohrbach, V., Pulvirenti, T., Adams, R., Ziemiecki, A. and Andres, A.C., 2009. Mammary epithelial-specific knockout of the ephrin-B2 gene leads to precocious epithelial cell death at lactation. Dev Growth Differ. 51, 809-19.
Wenger, R.H., 2002. Cellular adaptation to hypoxia: O2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O2-regulated gene expression. FASEB J. 16, 1151-62.
Wenzel, K., Daskalow, K., Herse, F., Seitz, S., Zacharias, U., Schenk, J.A., Schulz, H., Hubner, N., Micheel, B., Schlag, P.M., Osterziel, K.J., Ozcelik, C., Scherneck, S. and Jandrig, B., 2007. Expression of the protein phosphatase 1 inhibitor KEPI is downregulated in breast cancer cell lines and tissues and involved in the regulation of the tumor suppressor EGR1 via the MEK-ERK pathway. Biol Chem. 388, 489-95.
Wiesener, M.S., Turley, H., Allen, W.E., Willam, C., Eckardt, K.U., Talks, K.L., Wood, S.M., Gatter, K.C., Harris, A.L., Pugh, C.W., Ratcliffe, P.J. and Maxwell, P.H., 1998.

Induction of endothelial PAS domain protein-1 by hypoxia: characterization and comparison with hypoxia-inducible factor-1alpha. Blood. 92, 2260-8.
Wilson, P.A. and Hemmatibrivanlou, A., 1995. Induction of Epidermis and Inhibition of Neural Fate by Bmp-4. Nature. 376, 331-333.
Wilson, S.W. and Rubenstein, J.L., 2000. Induction and dorsoventral patterning of the telencephalon. Neuron. 28, 641-51.
Winberg, M.L., Noordermeer, J.N., Tamagnone, L., Comoglio, P.M., Spriggs, M.K., Tessier-Lavigne, M. and Goodman, C.S., 1998. Plexin A is a neuronal semaphorin receptor that controls axon guidance. Cell. 95, 903-16.
Winnier, G., Blessing, M., Labosky, P.A. and Hogan, B.L., 1995. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev. 9, 2105-16.
Wong, F., Welten, M.C., Anderson, C., Bain, A.A., Liu, J., Wicks, M.N., Pavlovska, G., Davey, M.G., Murphy, P., Davidson, D., Tickle, C.A., Stern, C.D., Baldock, R.A. and Burt, D.W., 2013. eChickAtlas: an introduction to the database. Genesis. 51, 365-71.
Woo, K., Shih, J. and Fraser, S.E., 1995. Fate maps of the zebrafish embryo. Curr Opin Genet Dev. 5, 439-43.
Wurst, W. and Bally-Cuif, L., 2001. Neural plate patterning: upstream and downstream of the isthmic organizer. Nat Rev Neurosci. 2, 99-108.
Xuan, S.H., Baptista, C.A., Balas, G., Tao, W.F., Soares, V.C. and Lai, E., 1995. Winged Helix Transcription Factor Bf-1 Is Essential for the Development of the Cerebral Hemispheres. Neuron. 14, 1141-1152.
Yamada, T., Pfaff, S.L., Edlund, T. and Jessell, T.M., 1993. Control of cell pattern in the neural tube: motor neuron induction by diffusible factors from notochord and floor plate. Cell. 73, 673-86.
Yamamoto, Y., Grubisic, K. and Oelgeschlager, M., 2007. Xenopus Tetraspanin-1 regulates gastrulation movements and neural differentiation in the early Xenopus embryo. Differentiation. 75, 235-45.
Yamauchi, J., Hirasawa, A., Miyamoto, Y., Kokubu, H., Nishii, H., Okamoto, M., Sugawara, Y., Tsujimoto, G. and Itoh, H., 2002. Role of Dbl's big sister in the anti-mitogenic pathway from alpha1B-adrenergic receptor to c-Jun N-terminal kinase. Biochem Biophys Res Commun. 296, 85-92.
Yang, M., Cagle, M.C. and Honig, M.G., 2010. Identification of cerebellin2 in chick and its preferential expression by subsets of developing sensory neurons and their targets in the dorsal horn. J Comp Neurol. 518, 2818-40.
Yang, Y., Drossopoulou, G., Chuang, P.T., Duprez, D., Marti, E., Bumcrot, D., Vargesson, N., Clarke, J., Niswander, L., McMahon, A. and Tickle, C., 1997. Relationship between dose, distance and time in Sonic Hedgehog-mediated regulation of anteroposterior polarity in the chick limb. Development. 124, 4393-4404.
Yasuo, H. and Lemaire, P., 2001. Role of Goosecoid, Xnot and Wnt antagonists in the maintenance of the notochord genetic programme in Xenopus gastrulae. Development. 128, 3783-93.
Yokouchi, Y., Vogan, K.J., Pearse, R.V., 2nd and Tabin, C.J., 1999. Antagonistic signaling by Caronte, a novel Cerberus-related gene, establishes left-right asymmetric gene expression. Cell. 98, 573-83.
Yoshioka, H., Meno, C., Koshiba, K., Sugihara, M., Itoh, H., Ishimaru, Y., Inoue, T., Ohuchi, H., Semina, E.V., Murray, J.C., Hamada, H. and Noji, S., 1998. Pitx2, a bicoid-type homeobox gene, is involved in a lefty-signaling pathway in determination of left-right asymmetry. Cell. 94, 299-305.

Yuan, S., Darnell, D.K. and Schoenwolf, G.C., 1995. Mesodermal patterning during avian gastrulation and neurulation: experimental induction of notochord from non-notochordal precursor cells. Dev Genet. 17, 38-54.

Yunta, M. and Lazo, P.A., 2003. Tetraspanin proteins as organisers of membrane microdomains and signalling complexes. Cell Signal. 15, 559-64.

Zacchigna, S., Oh, H., Wilsch-Brauninger, M., Missol-Kolka, E., Jaszai, J., Jansen, S., Tanimoto, N., Tonagel, F., Seeliger, M., Huttner, W.B., Corbeil, D., Dewerchin, M., Vinckier, S., Moons, L. and Carmeliet, P., 2009. Loss of the cholesterol-binding protein prominin-1/CD133 causes disk dysmorphogenesis and photoreceptor degeneration. J Neurosci. 29, 2297-308.

Zeller, R., Lopez-Rios, J. and Zuniga, A., 2009. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. Nat Rev Genet. 10, 845-58.

Zeltser, L.M., 2005. Shh-dependent formation of the ZLI is opposed by signals from the dorsal diencephalon. Development. 132, 2023-2033.

Zerial, M. and McBride, H., 2001. Rab proteins as membrane organizers. Nat Rev Mol Cell Biol. 2, 107-17.

Zerucha, T., Stuhmer, T., Hatch, G., Park, B.K., Long, Q., Yu, G., Gambarotta, A., Schultz, J.R., Rubenstein, J.L. and Ekker, M., 2000. A highly conserved enhancer in the Dlx5/Dlx6 intergenic region is the site of cross-regulatory interactions between Dlx genes in the embryonic forebrain. J Neurosci. 20, 709-21.

Zhu, L., Belo, J.A., De Robertis, E.M. and Stern, C.D., 1999a. Goosecoid regulates the neural inducing strength of the mouse node. Dev Biol. 216, 276-81.

Zhu, L., Marvin, M.J., Gardiner, A., Lassar, A.B., Mercola, M., Stern, C.D. and Levin, M., 1999b. Cerberus regulates left-right asymmetry of the embryonic head and heart. Curr Biol. 9, 931-8.

Zimmerman, L.B., DeJesusEscobar, J.M. and Harland, R.M., 1996. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. Cell. 86, 599-606.

Zimmermann, H., 1999. Two novel families of ectonucleotidases: molecular structures, catalytic properties and a search for function. Trends Pharmacol Sci. 20, 231-6.

Zou, Y.R., Kottmann, A.H., Kuroda, M., Taniuchi, I. and Littman, D.R., 1998. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 393, 595-9.

Appendix

1. **Whole-mount *in-situ* hybridisation data**

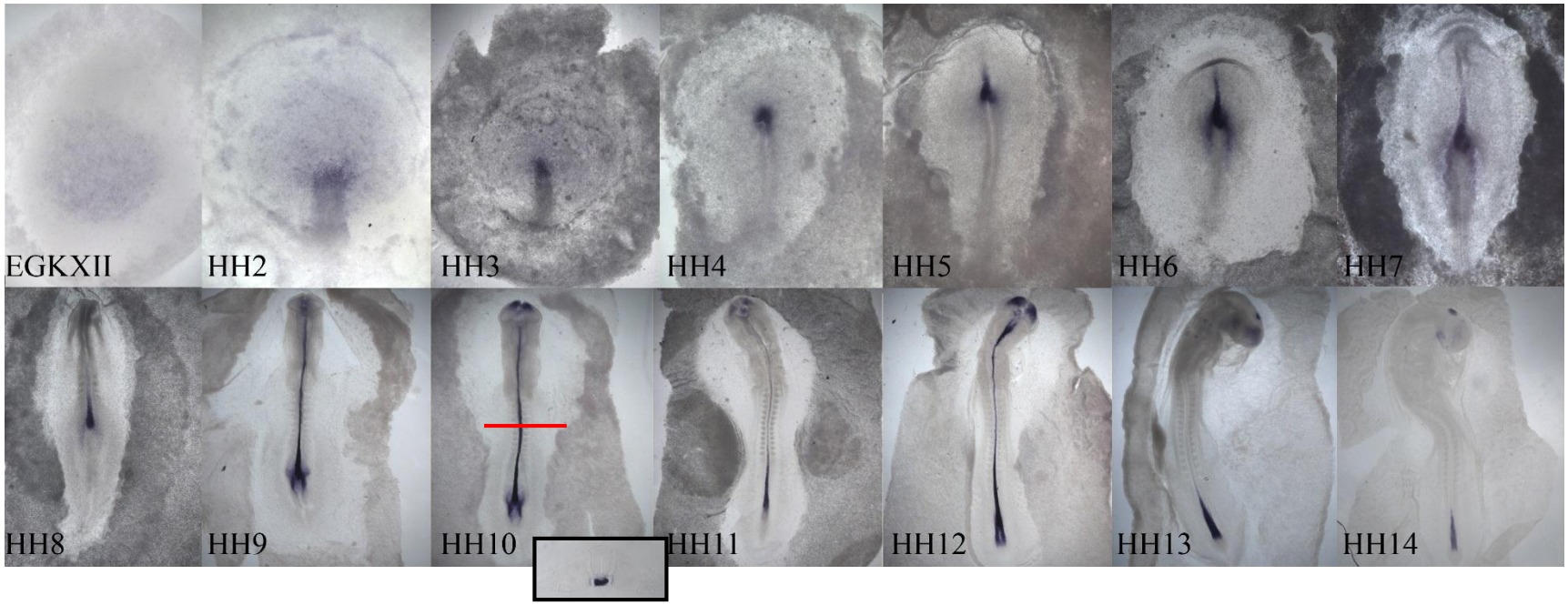


Figure.1.1 cNOT1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from dorsal view.

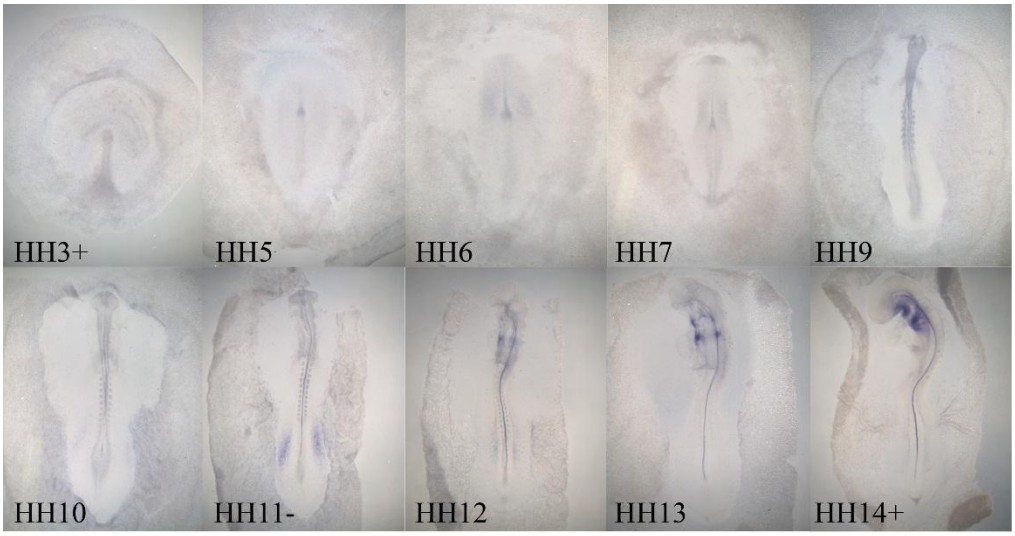


Figure 1.2 LMO7 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from dorsal view. Data kindly provided by Dr. Claire Anderson.

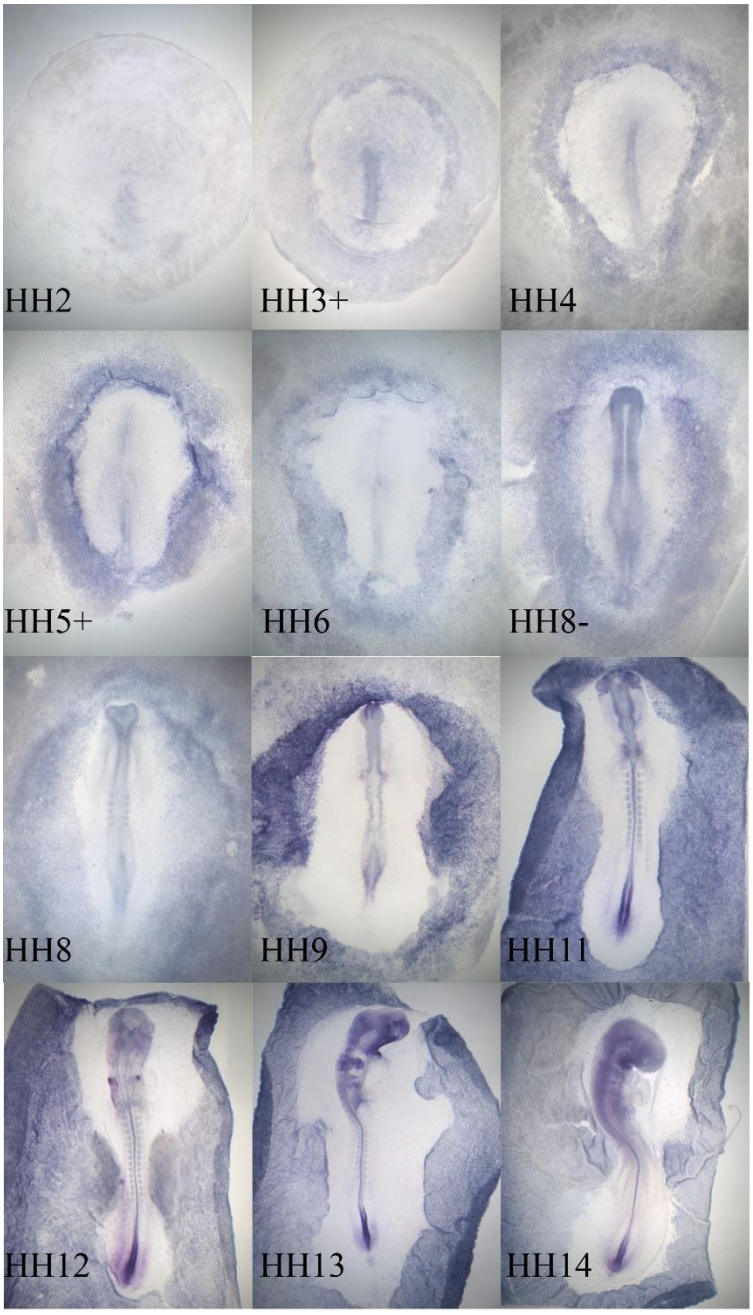


Figure 1.3 GNPDA1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

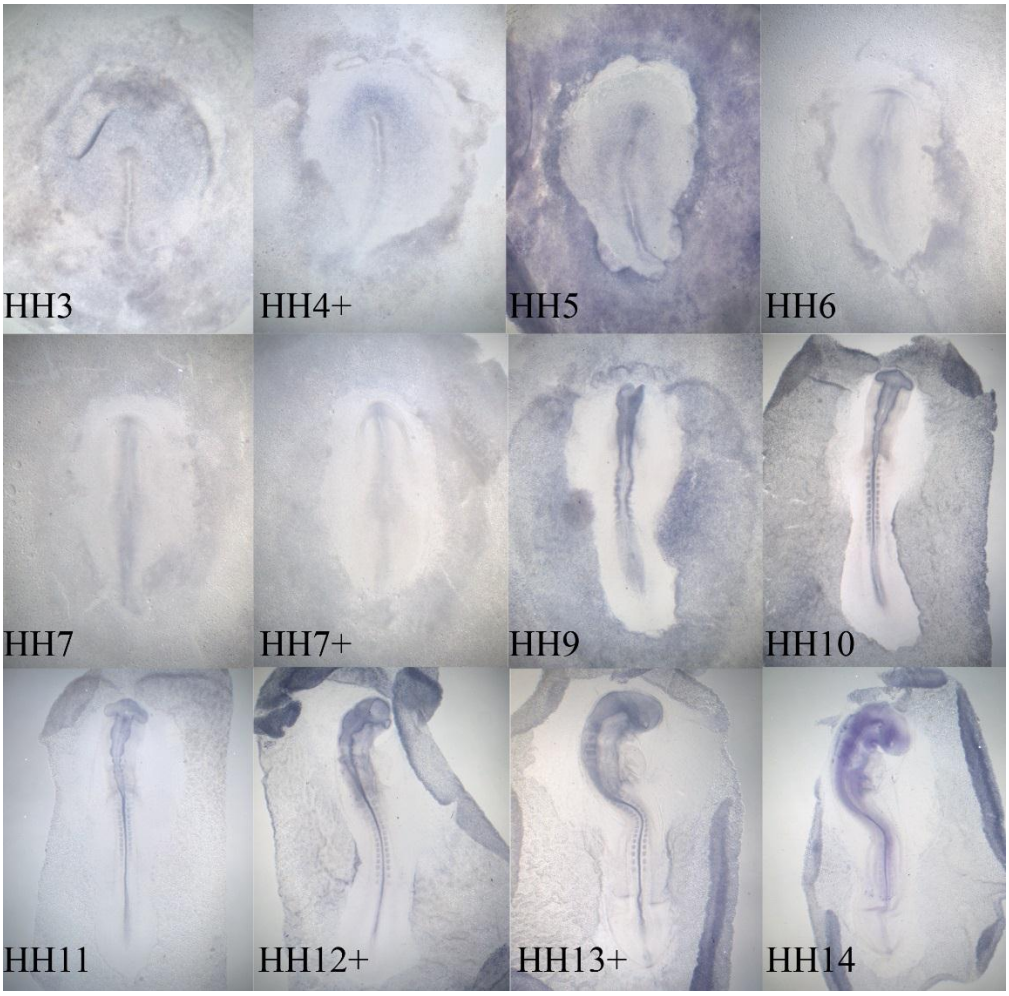


Figure 1.4 KCNMA1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

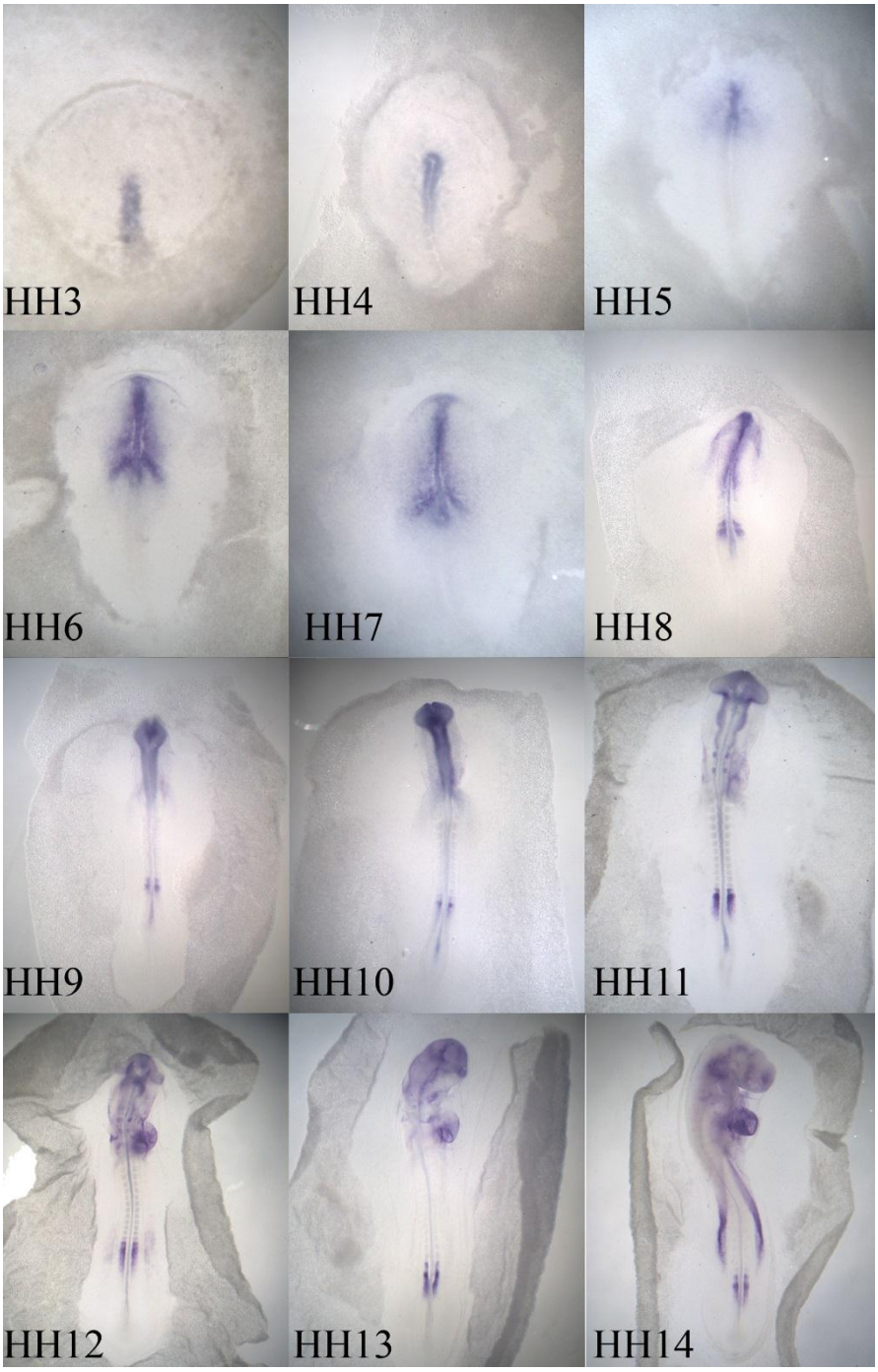


Figure 1.5 KIRREL3 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

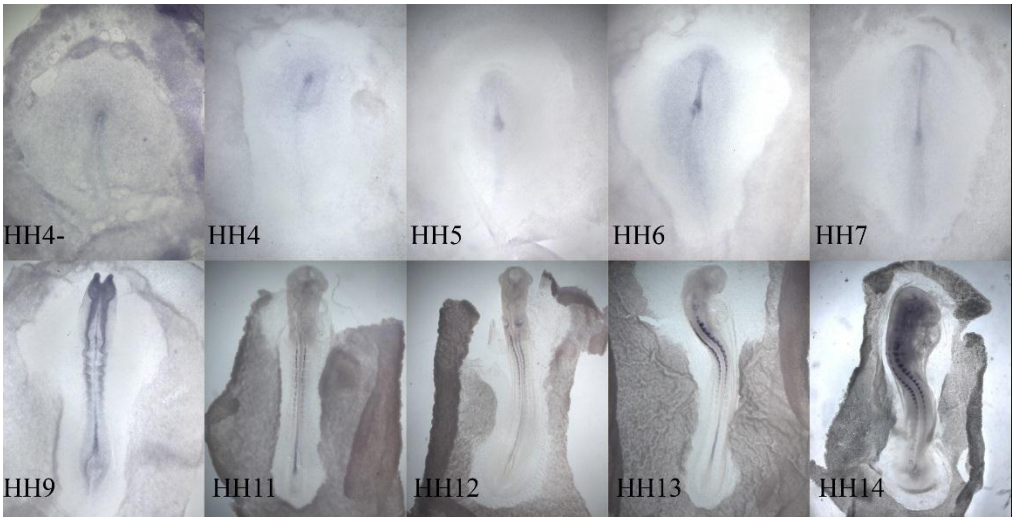


Figure 1.6 MCF2L expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view.

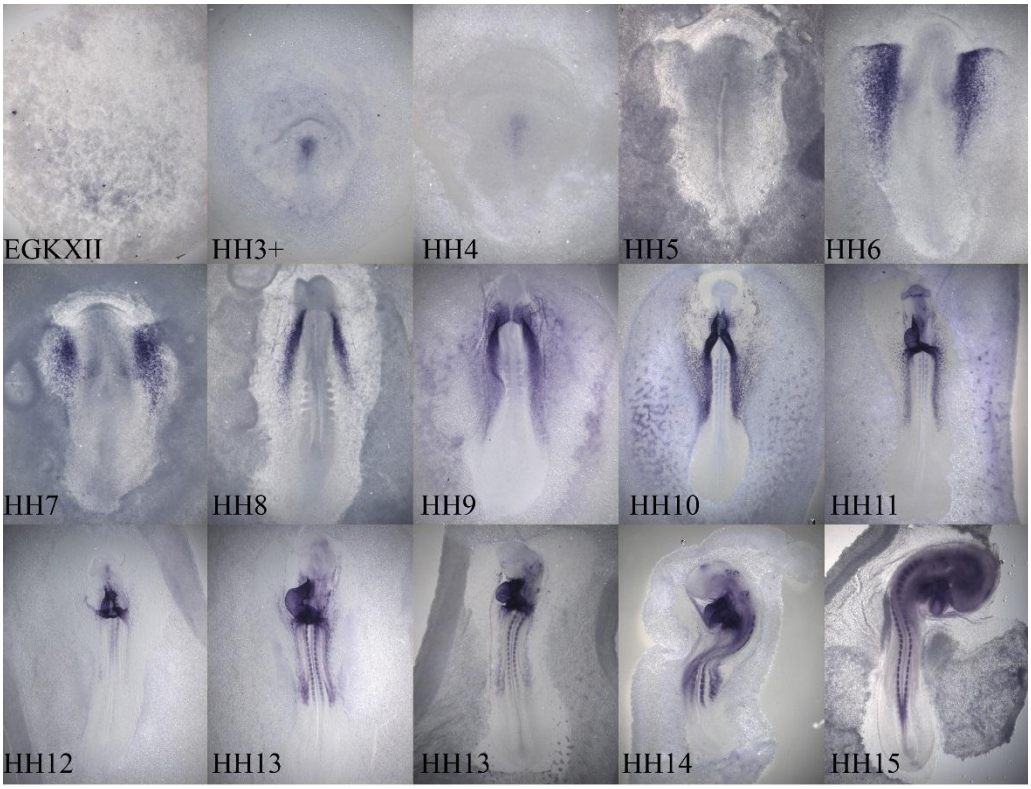


Figure 1.7 PKIγ expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view except HH15 which is from the dorsal view. Data kindly provided by Dr. Claire Anderson.

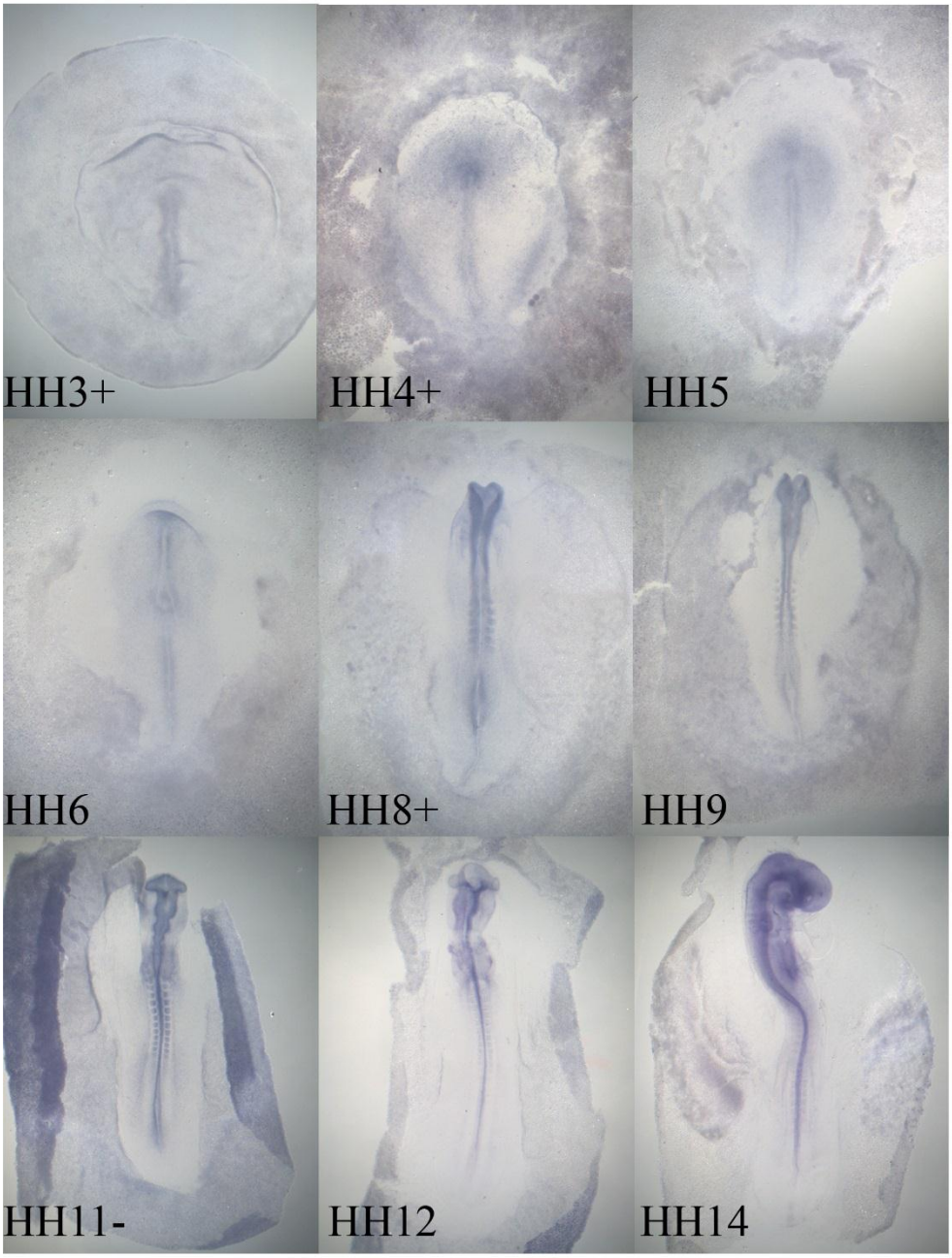


Figure 1.8 PLK1S1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

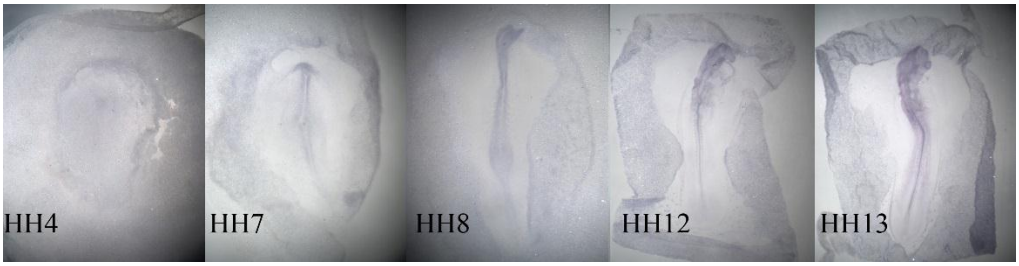


Figure 1.9 PIK3CD expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

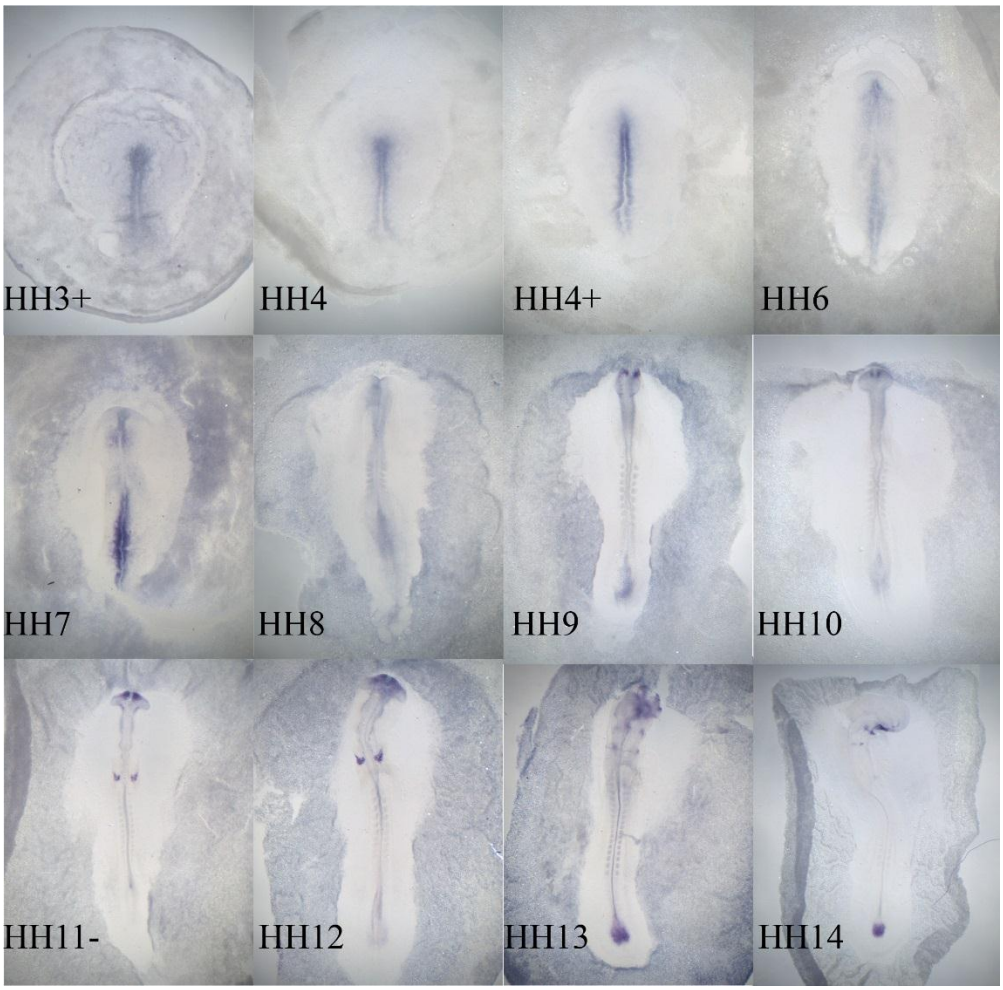


Figure 1.10 PIK3R5 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

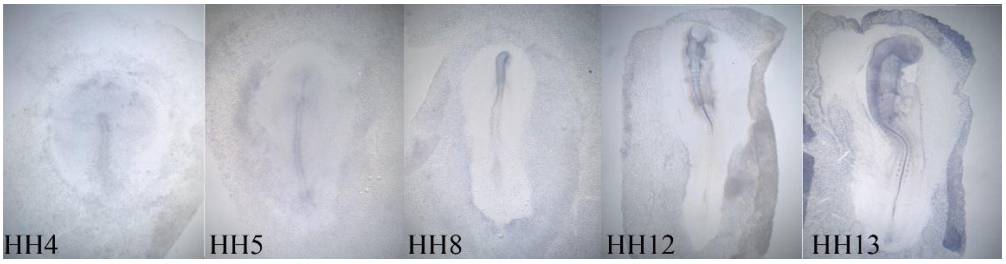


Figure 1.11 PPP1R14C expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

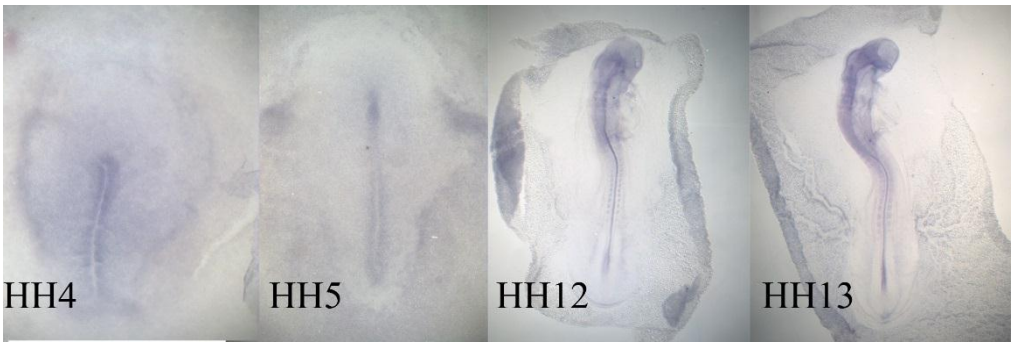


Figure 1.12 SLC39A11 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

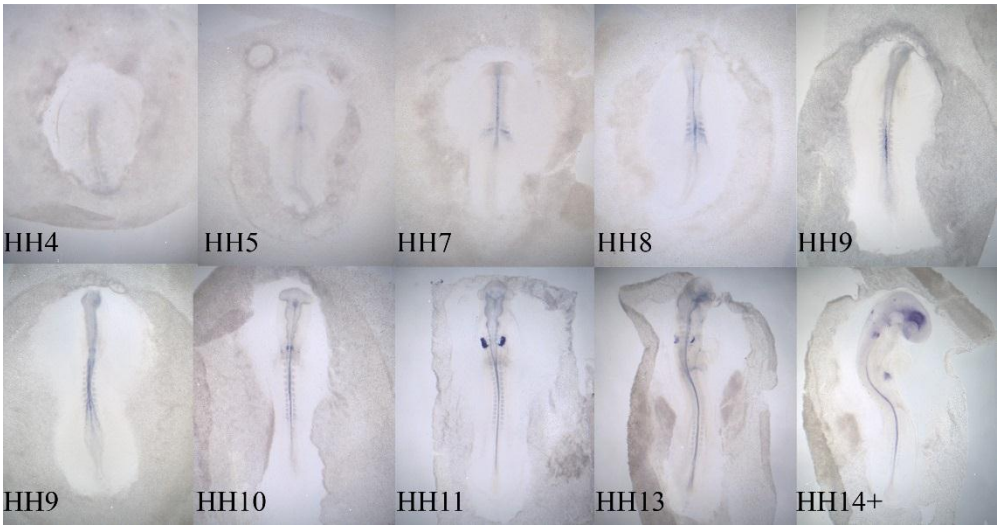


Figure 1.13 ADRA2A expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

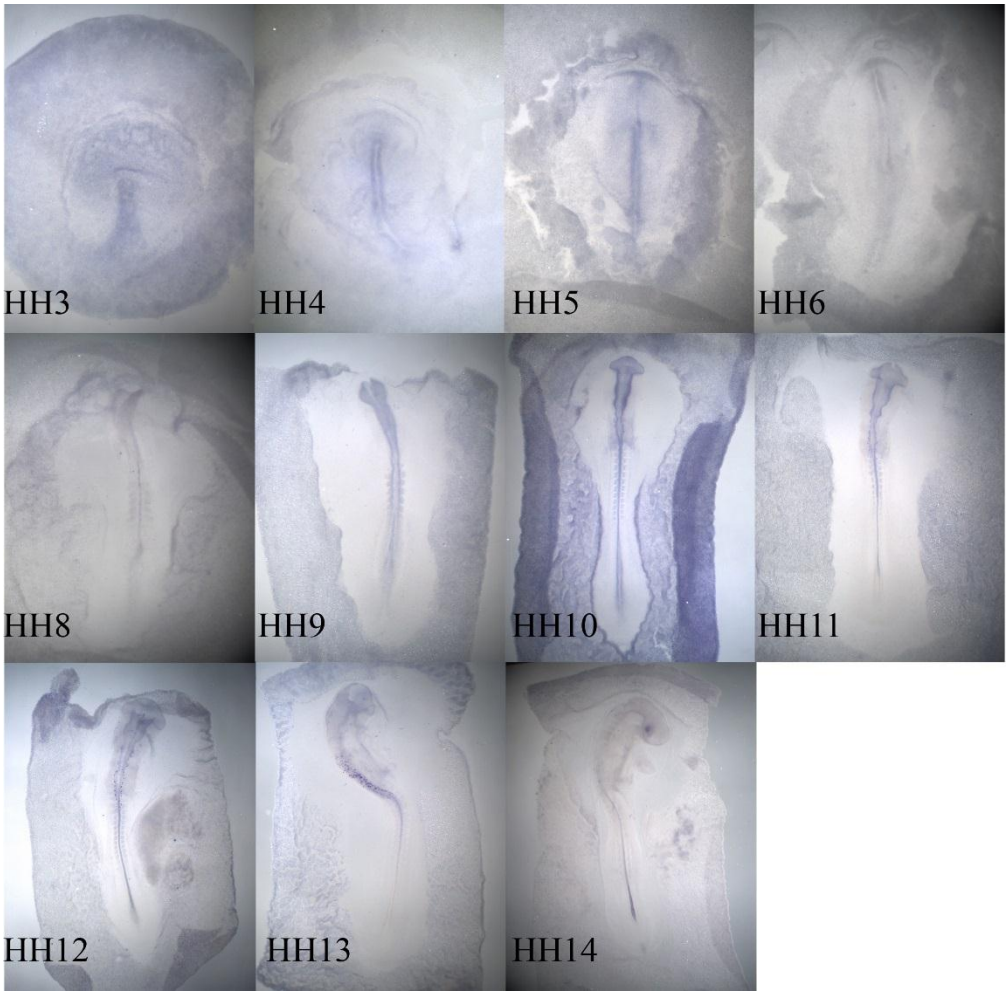


Figure 1.14 CHGA expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

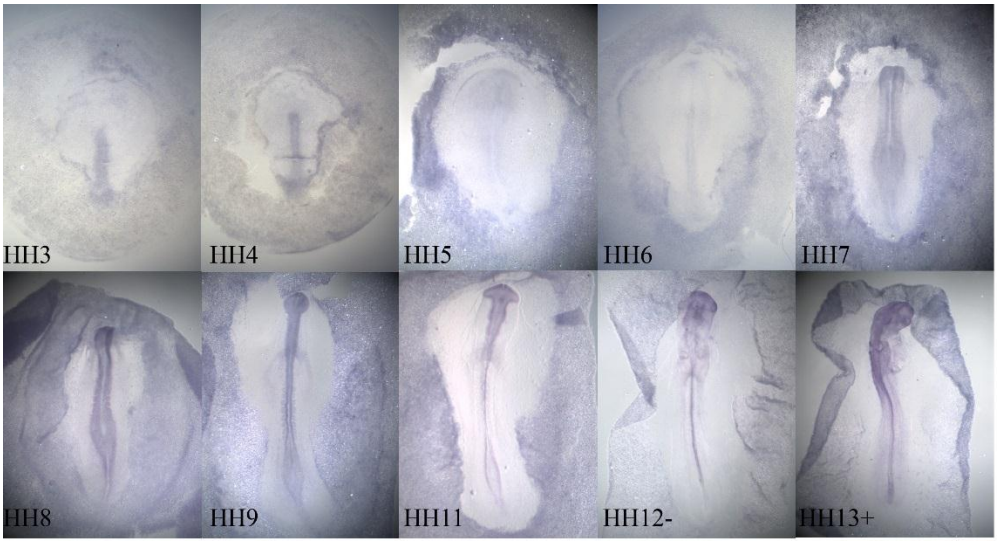


Figure 1.15 ENPP4 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

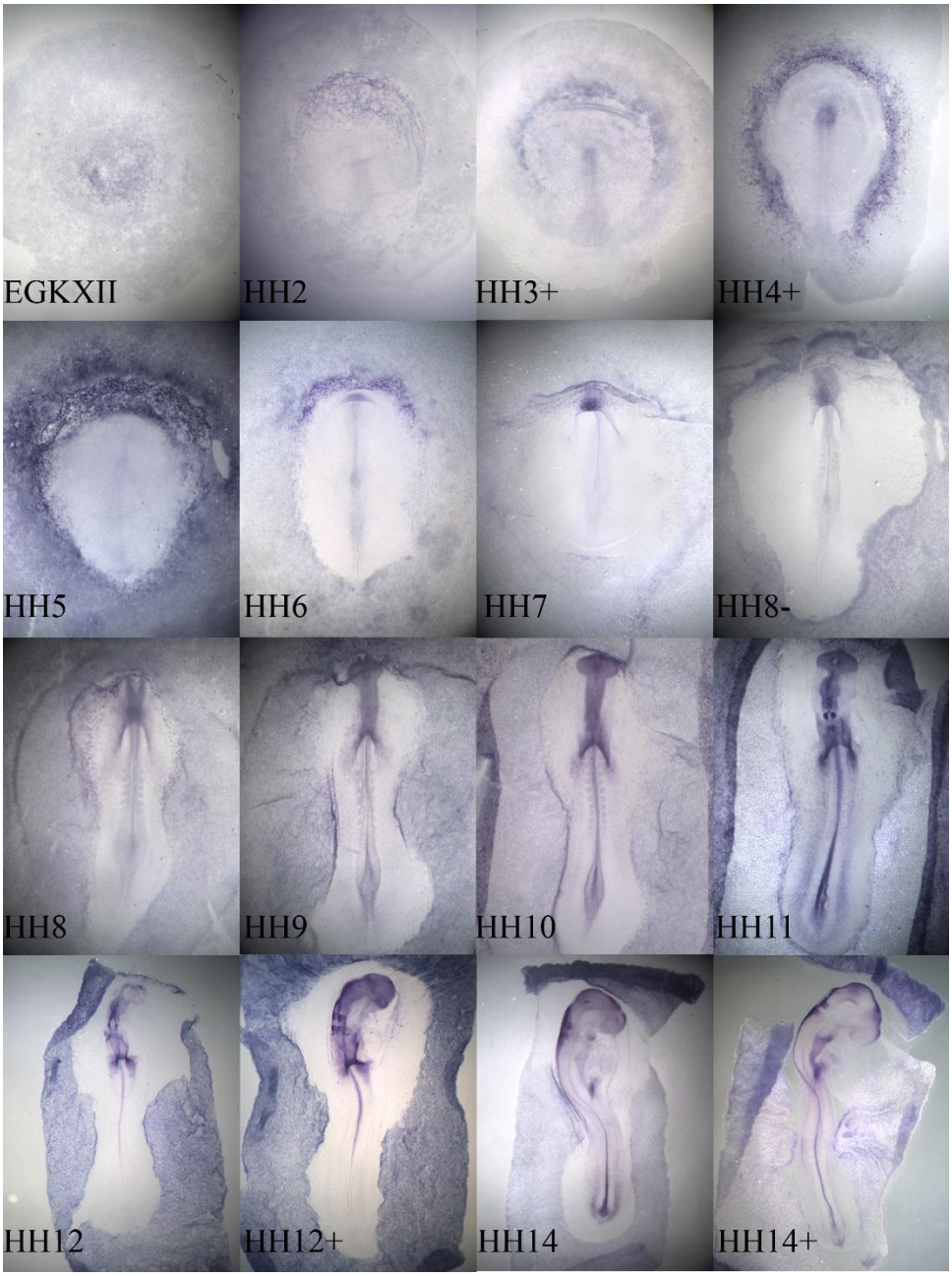


Figure 1.16 FBLN7L expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

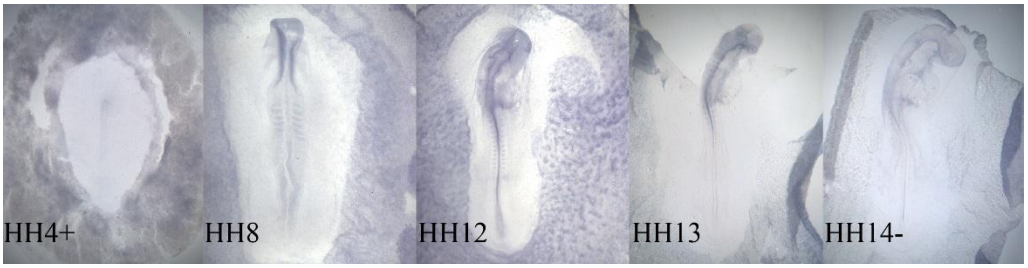


Figure 1.17 NXPH1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data of HH4+, 13 and 14- kindly provided by Dr. Claire Anderson.

Figure 1.18 NRP1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view. Data kindly provided by Dr. Claire Anderson.

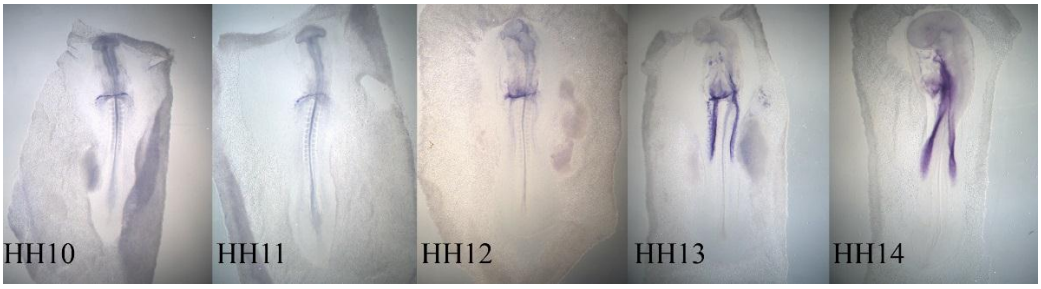


Figure 1.19 NRSN1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view. Data kindly provided by Dr. Claire Anderson.

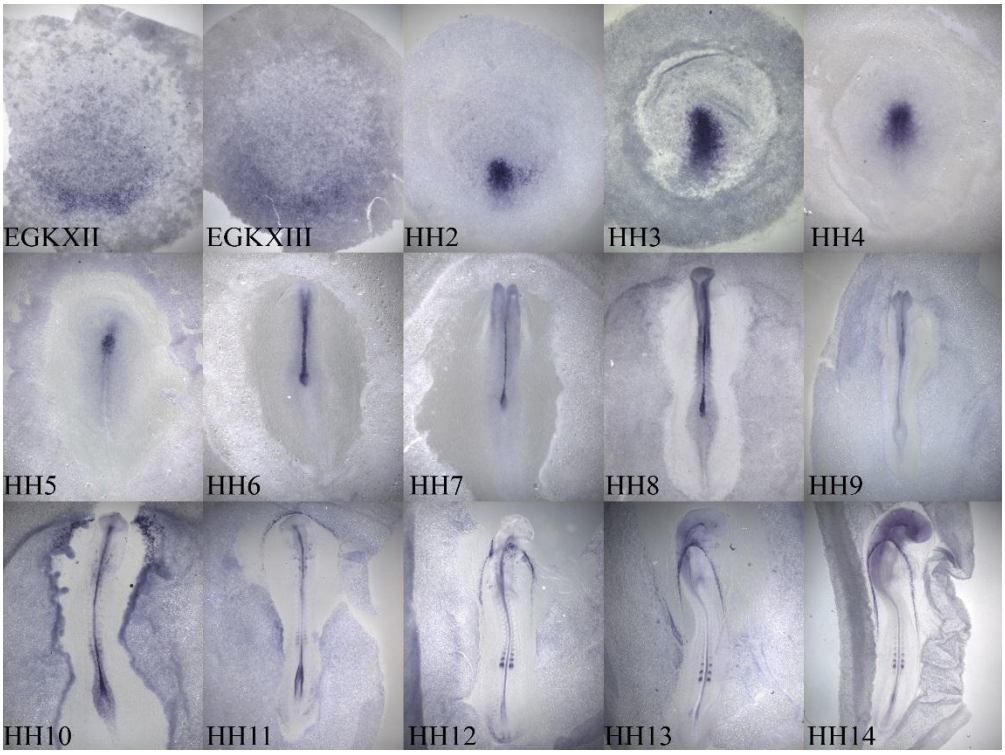


Figure 1.20 PCSK6 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

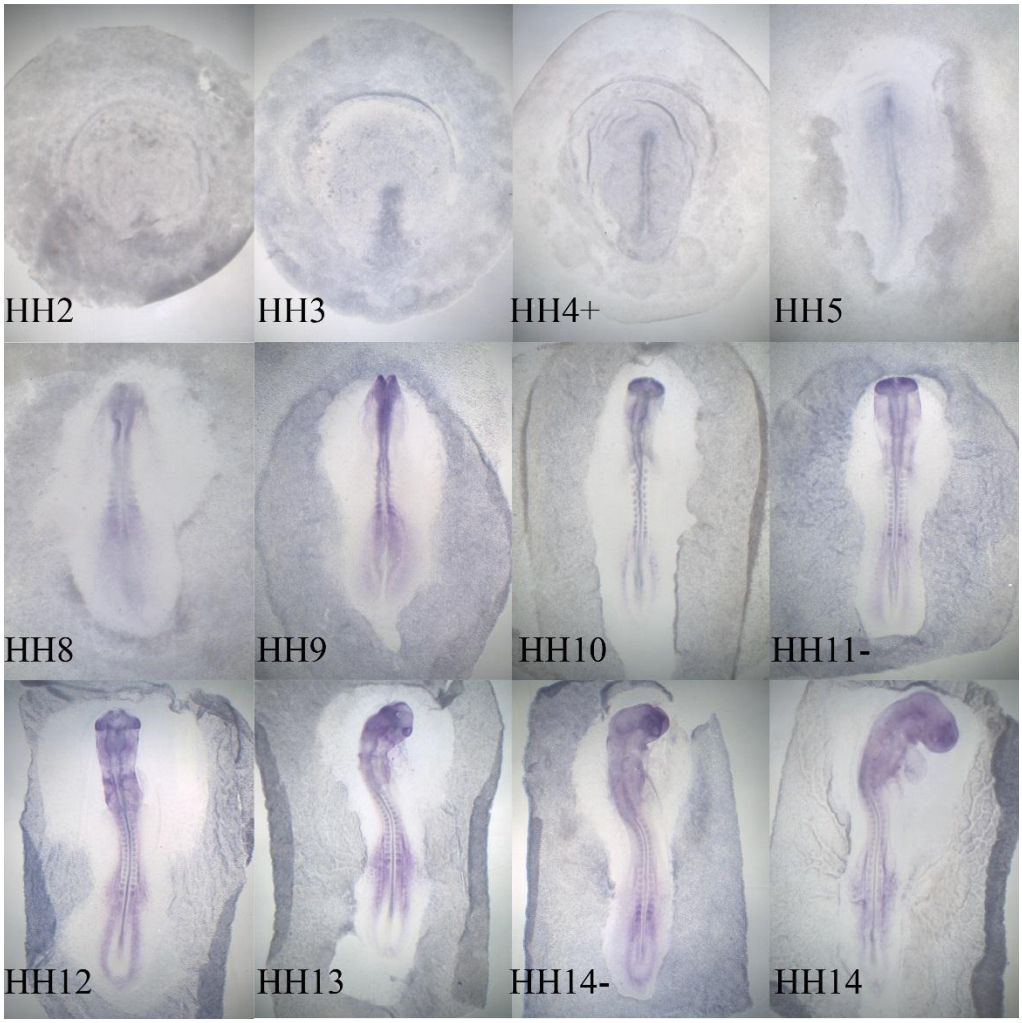


Figure 1.21 PRKG1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

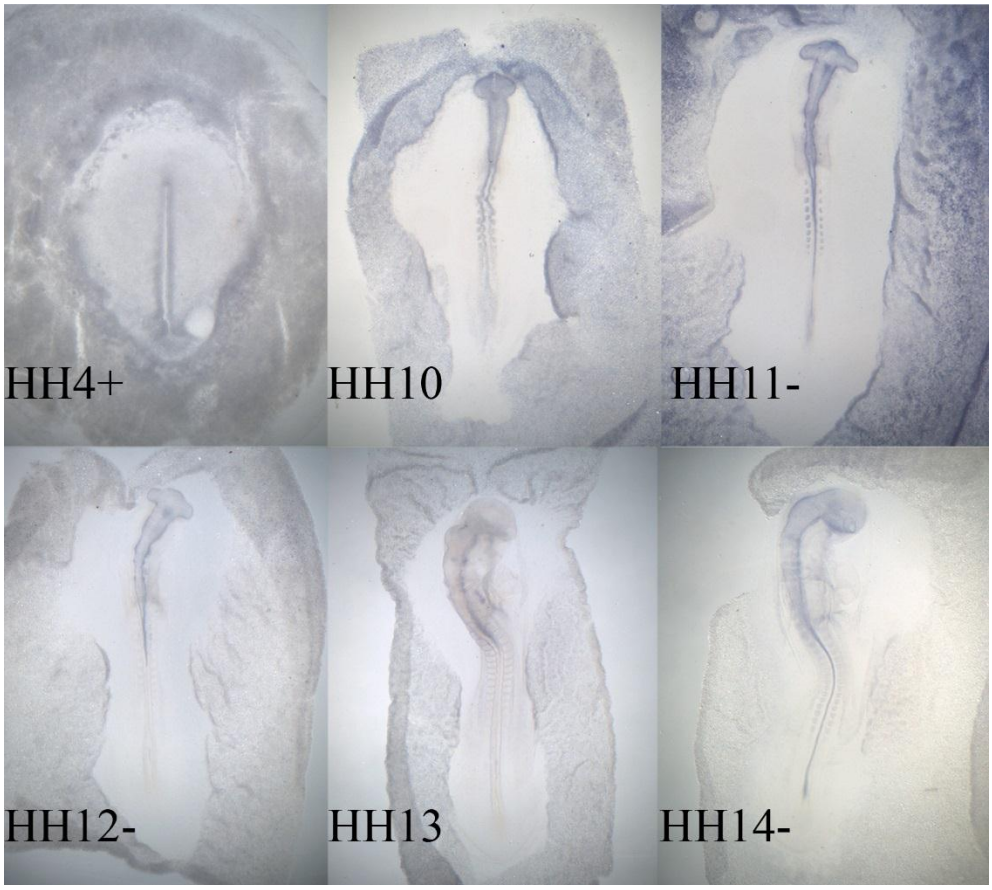


Figure 1.22 PROM1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

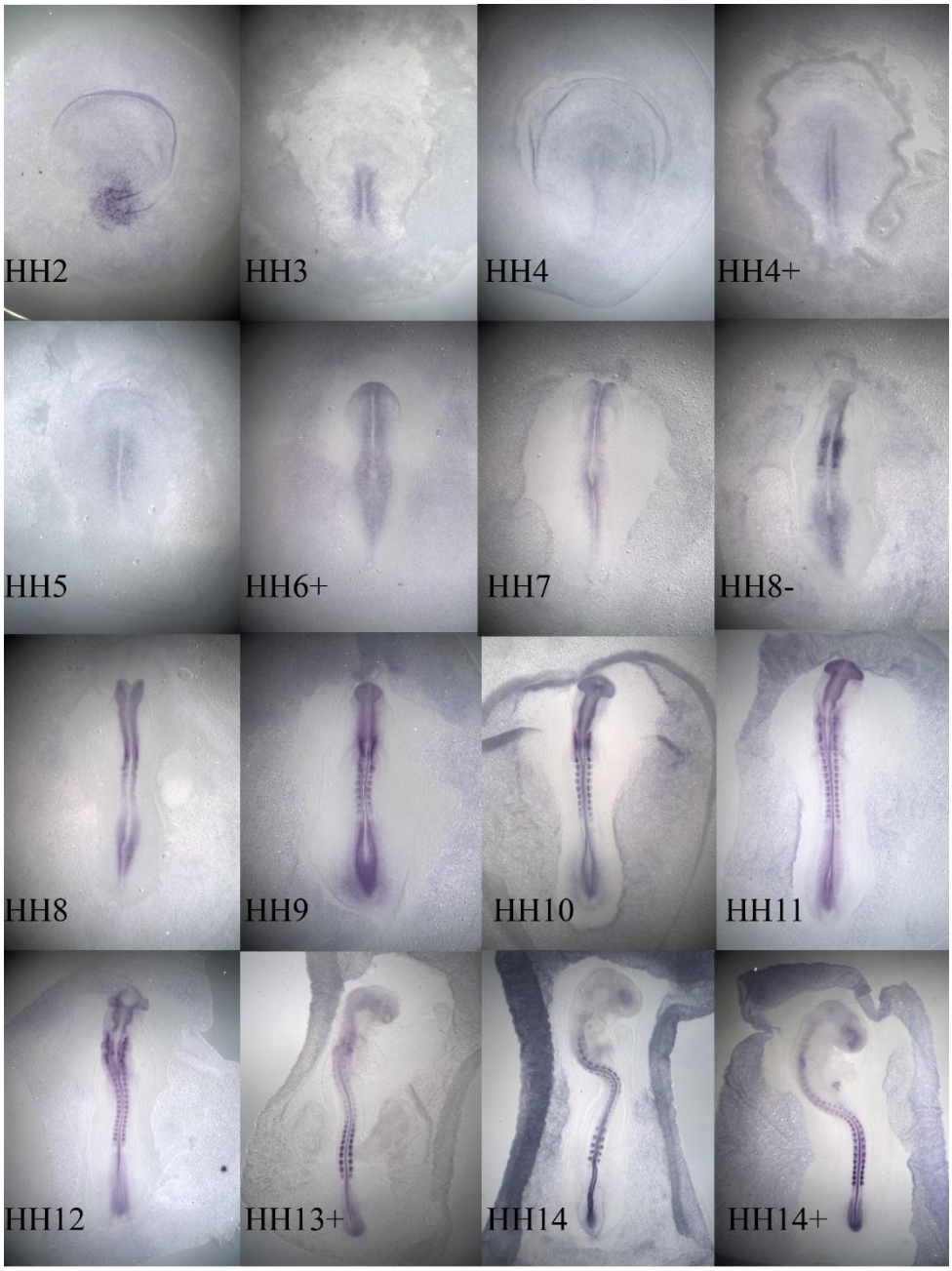


Figure 1.23 RAB11-FIP4 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

Figure 1.24 SMOC1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

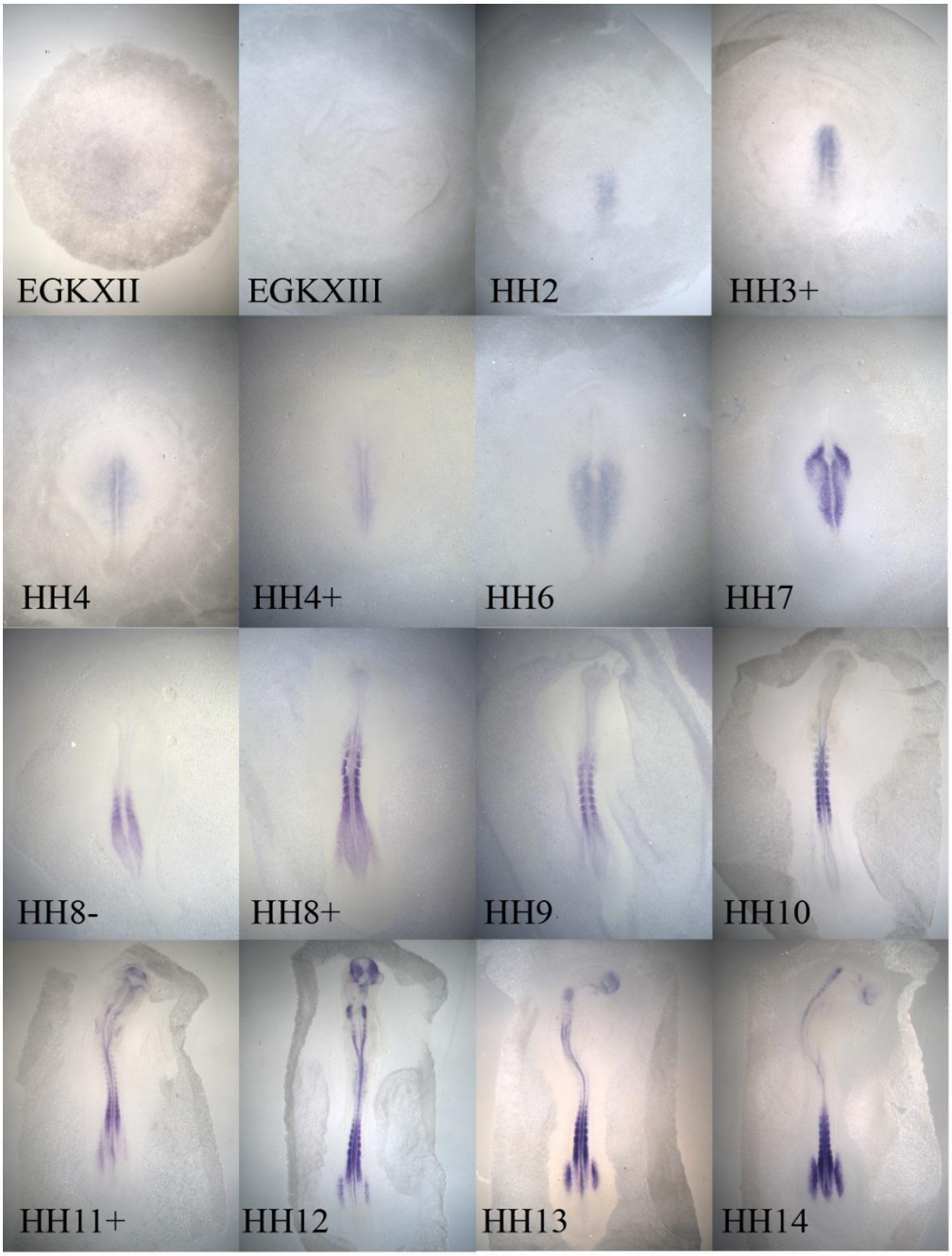


Figure 1.25 ChEST21m16 expression was studied by whole-mount *in-situ* hybridisation. This is another probe staining for SMOC1 as well. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

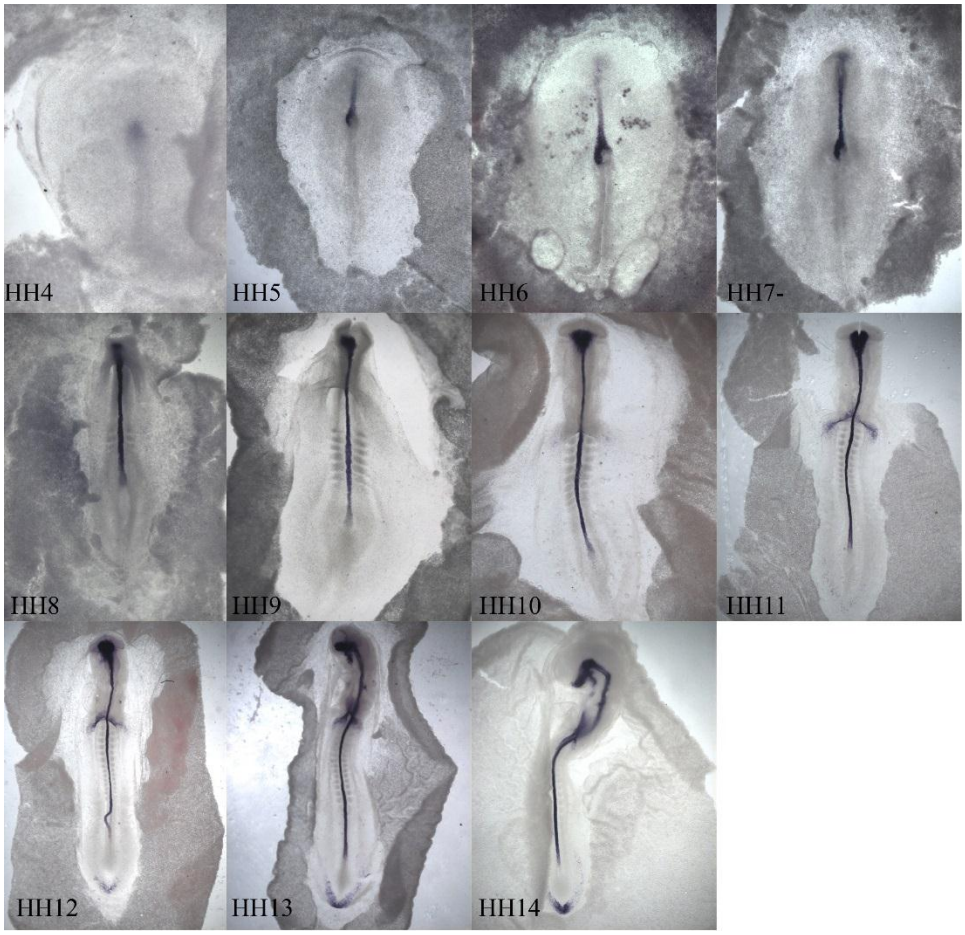


Figure 1.26 SHH expression was studied by whole-mount *in-situ* hybridisation. Pictures from HH4 to HH7 were taken from dorsal view.

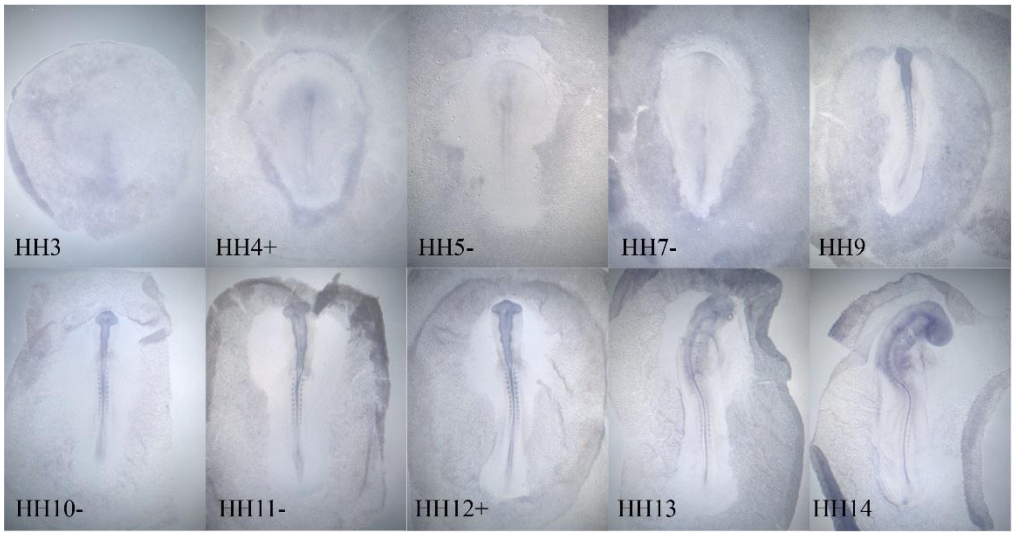


Figure 1.27 TMEM63C expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

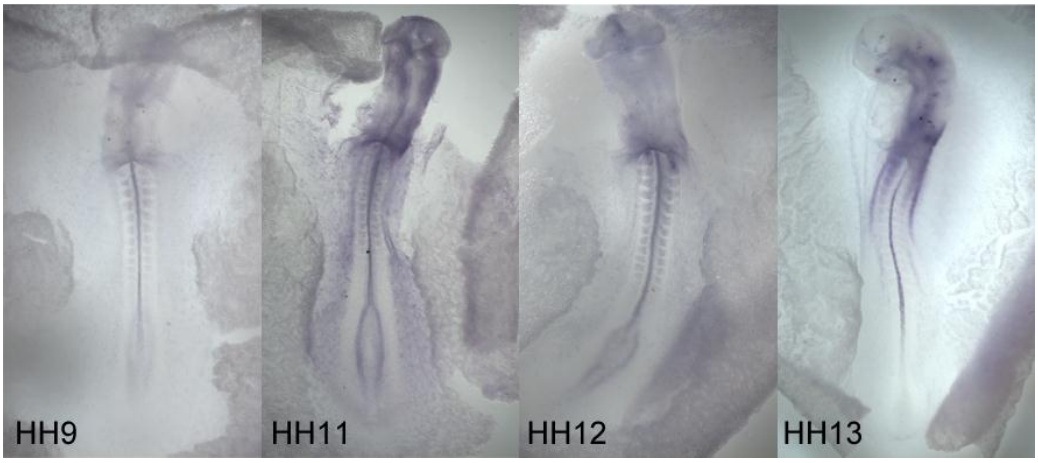


Figure 1.28 TMTC2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view.

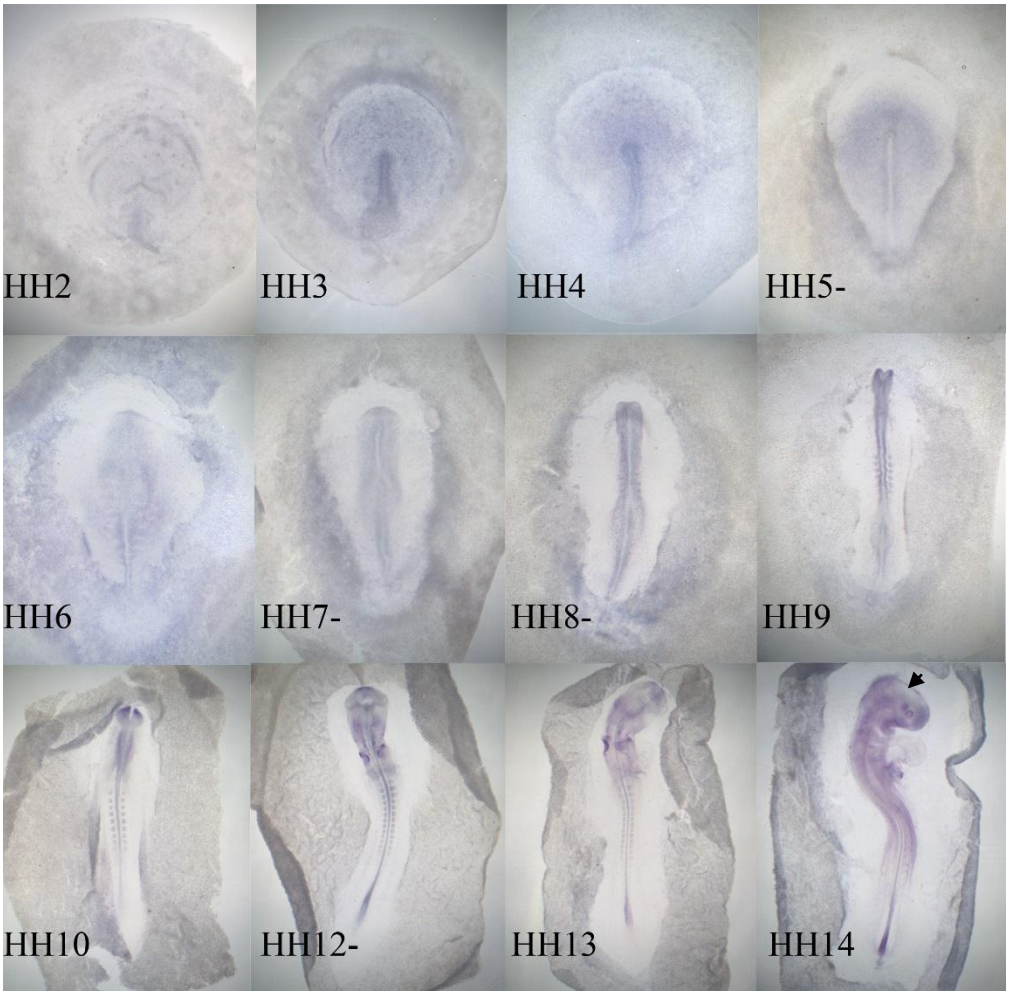


Figure 1.29 TSPAN6 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

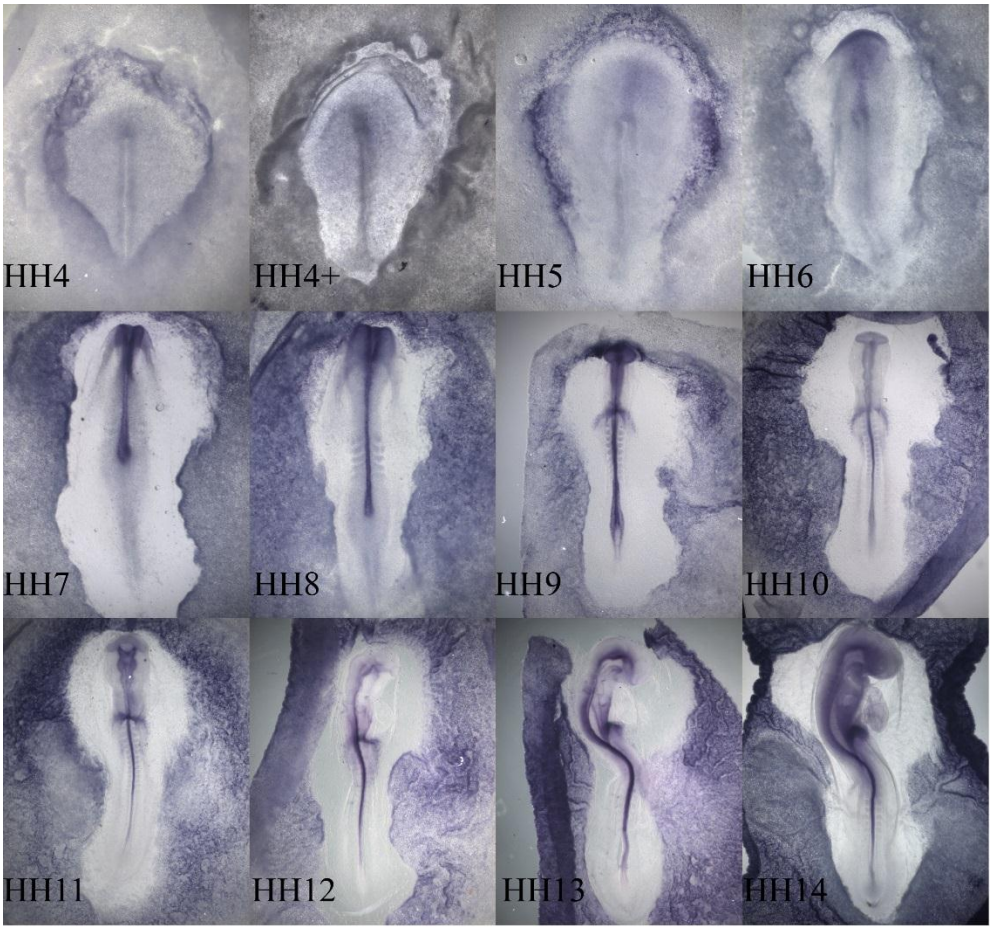


Figure 1.30 VTN expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

Figure 1.31 ChEST125i5 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

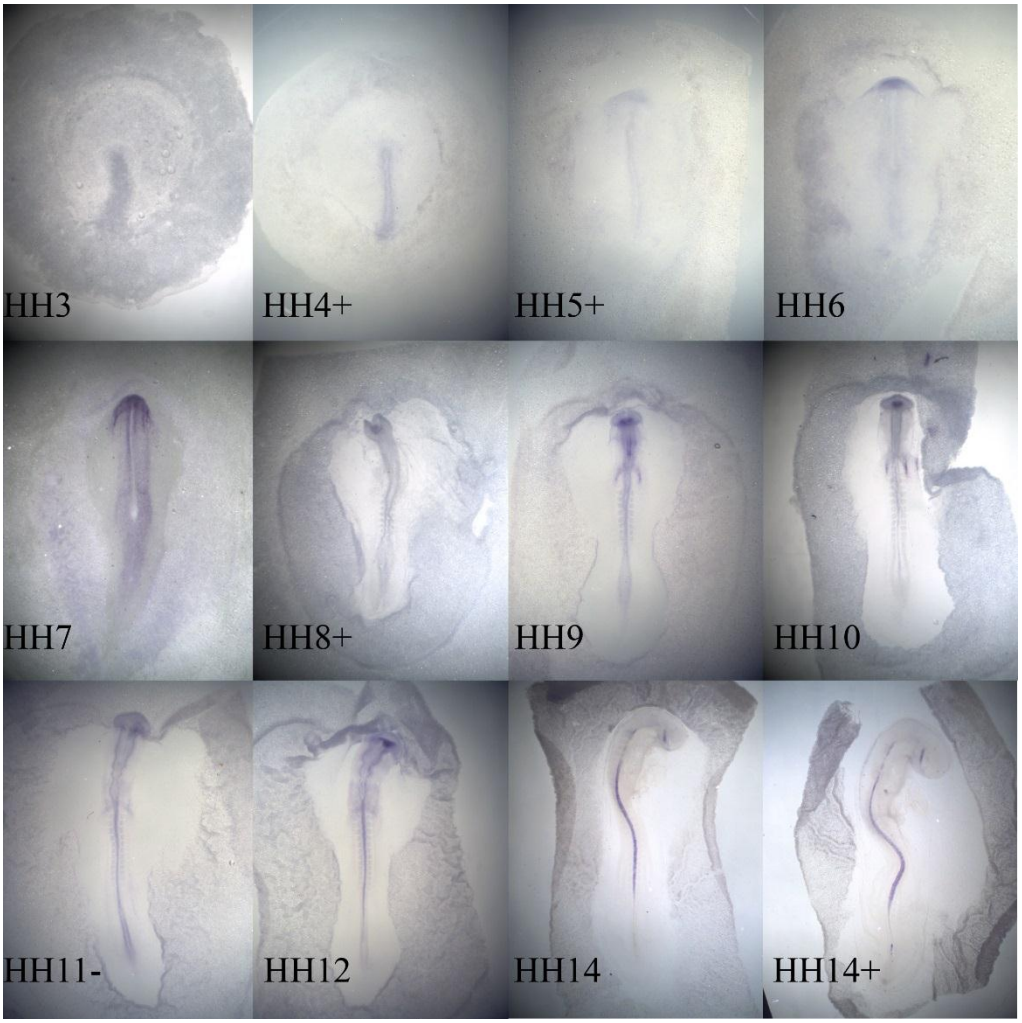


Figure 1.32 ChEST851k1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. The expression pattern is as described in text. Data kindly provided by Dr. Claire Anderson.

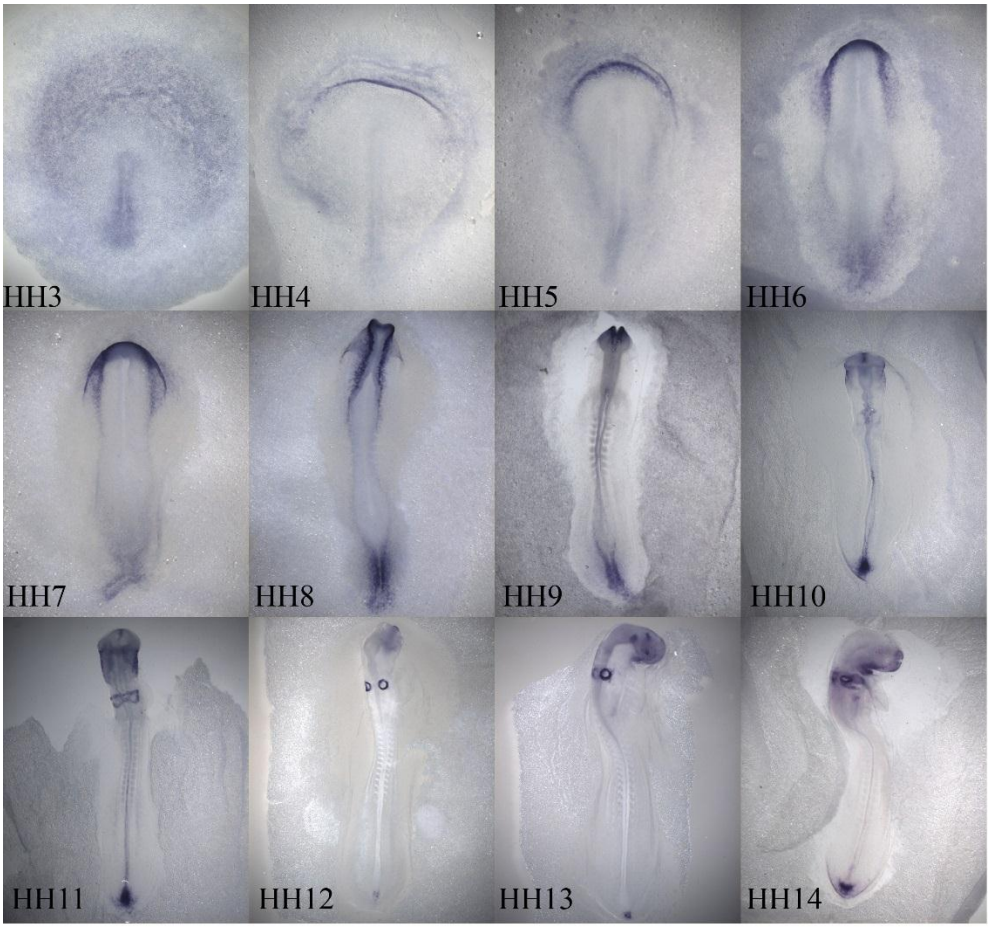


Figure 1.33 DLX5 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

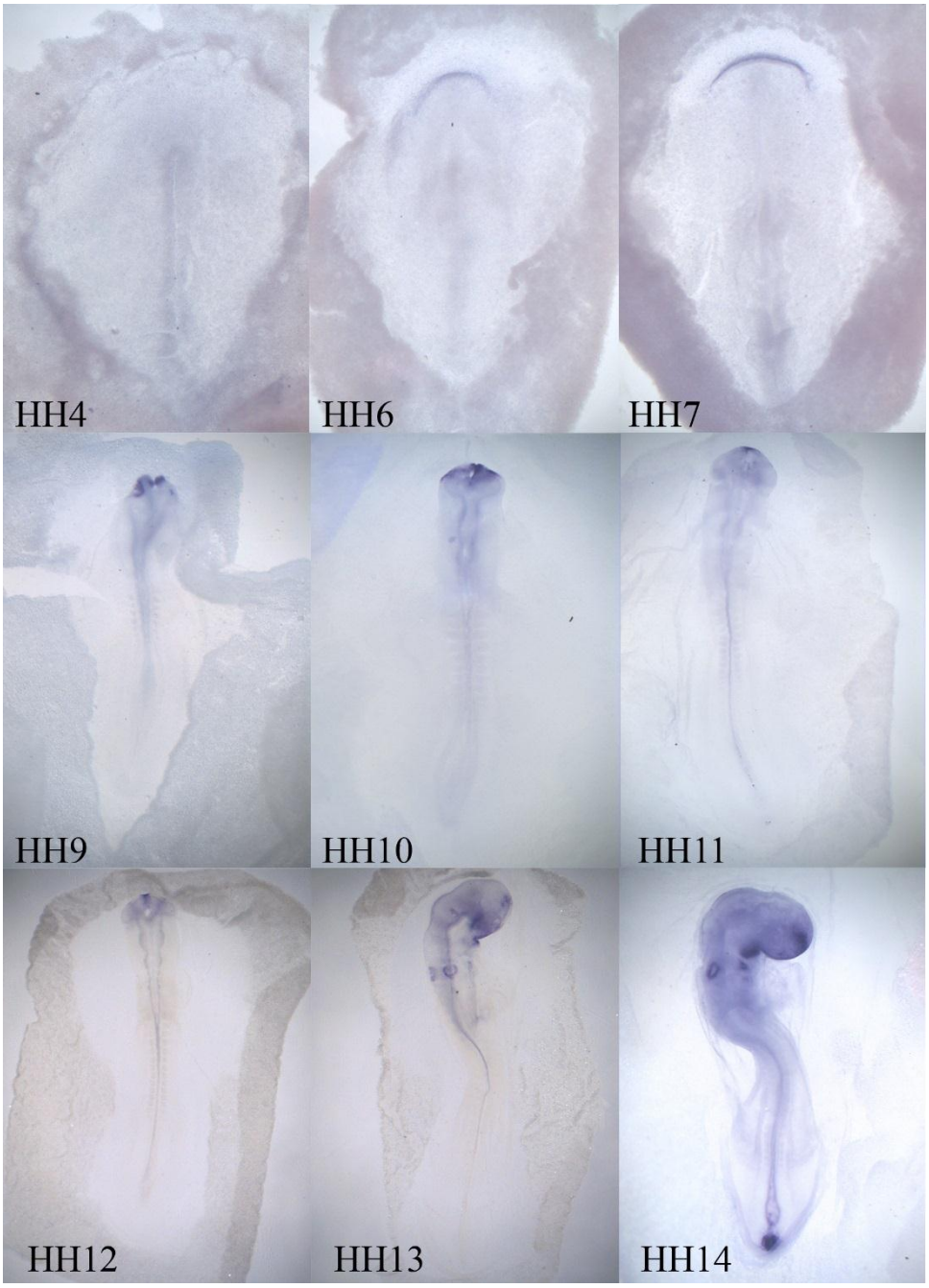


Figure 1.34 DLX6 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view.

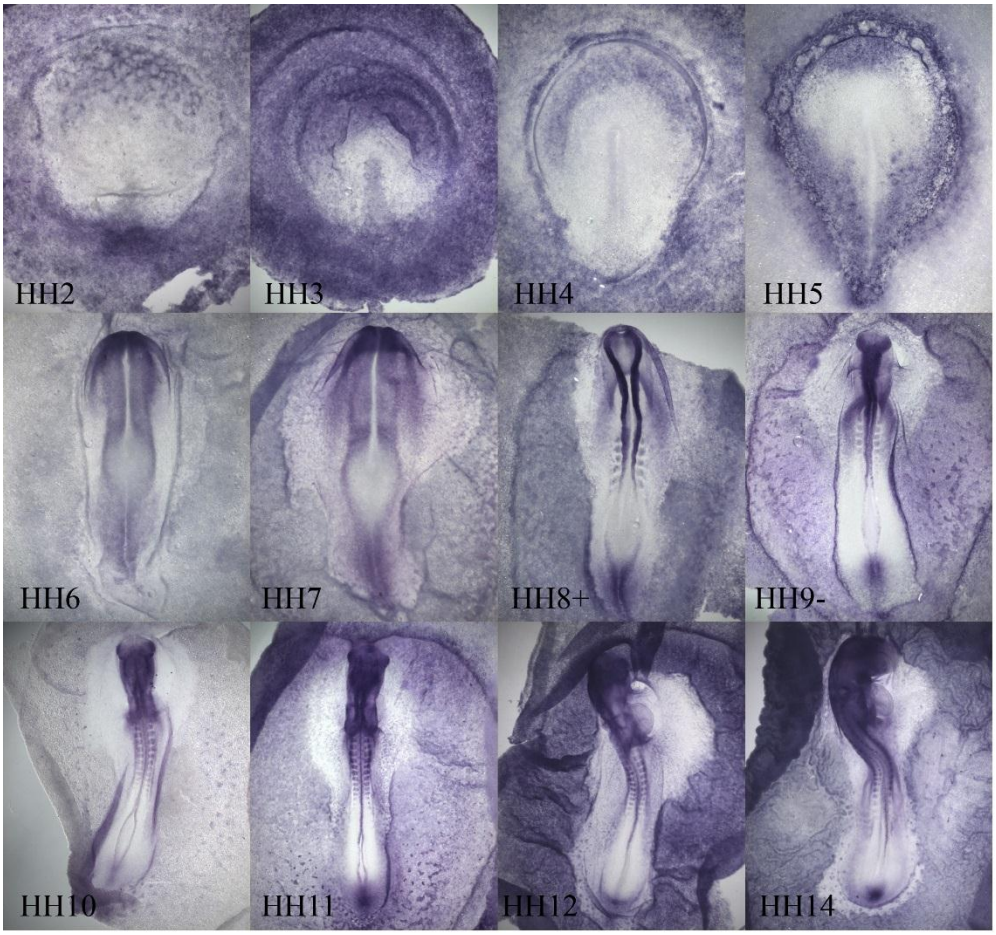


Figure 1.35 ID2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

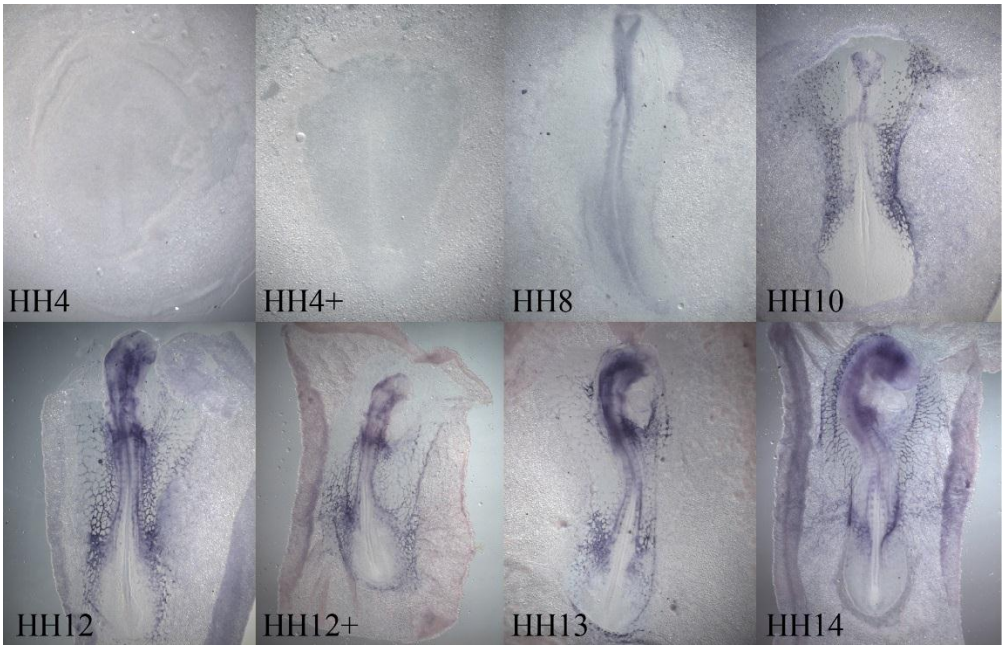


Figure 1.36 LDB2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

Figure 1.37 Msx1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

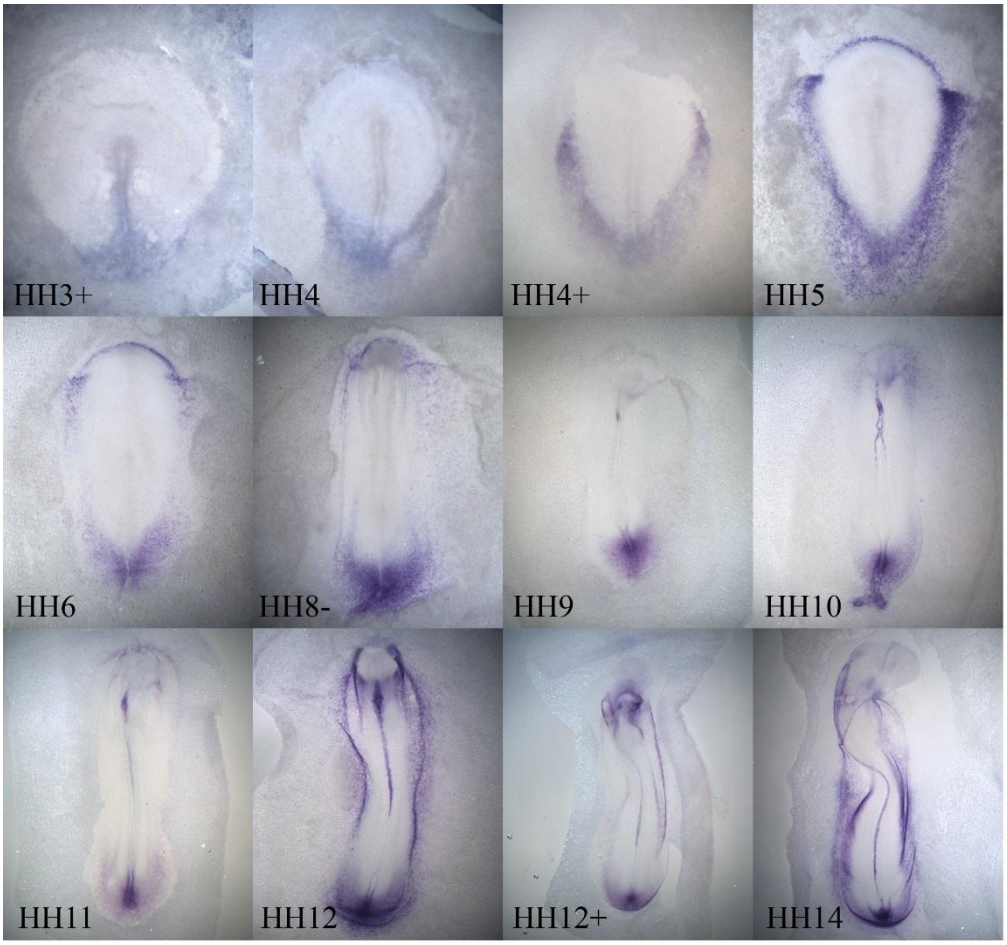


Figure 1.38 Msx2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

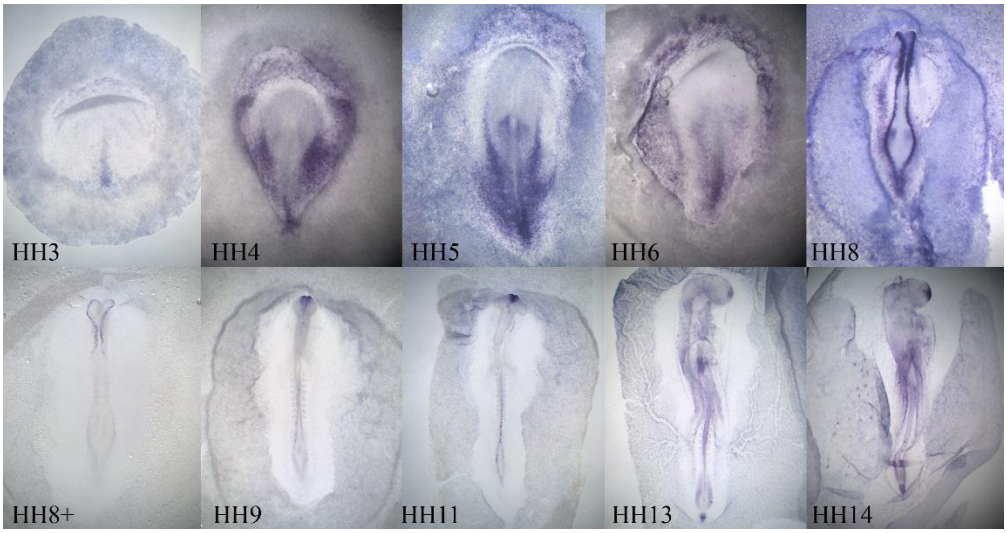


Figure 1.39 BTG2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data for HH3, 8+, 9, 11 and 13 kindly provided by Dr. Claire Anderson.

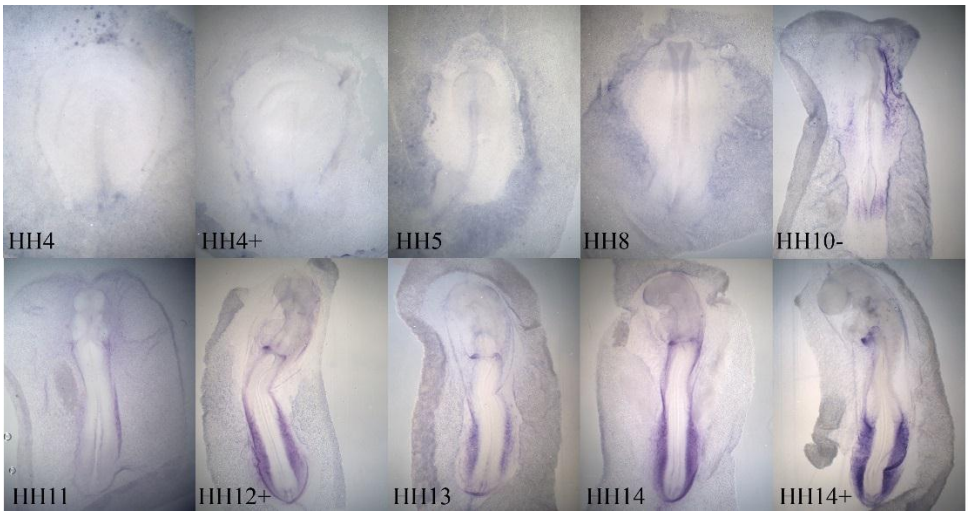


Figure 1.40 DSP expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view. Data kindly provided by Dr. Claire Anderson.

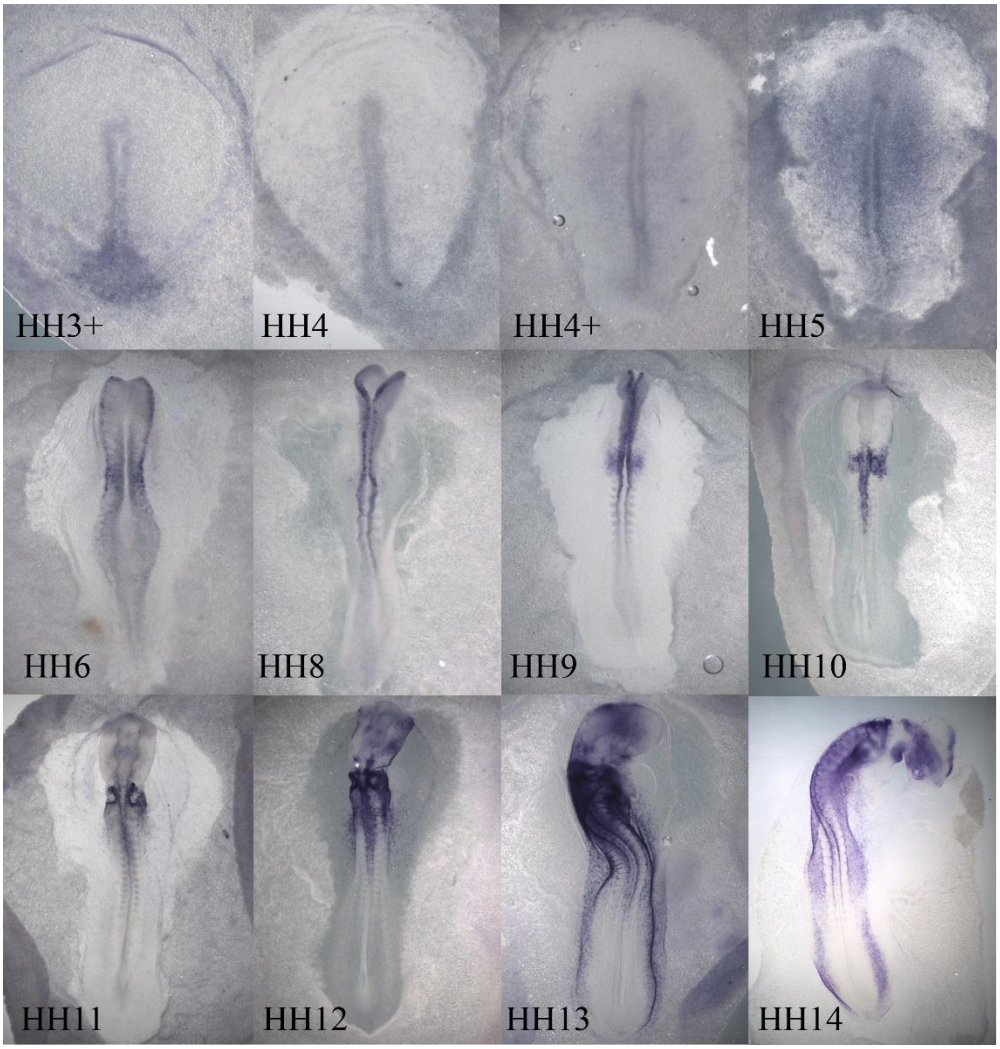


Figure 1.41 MOXD1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

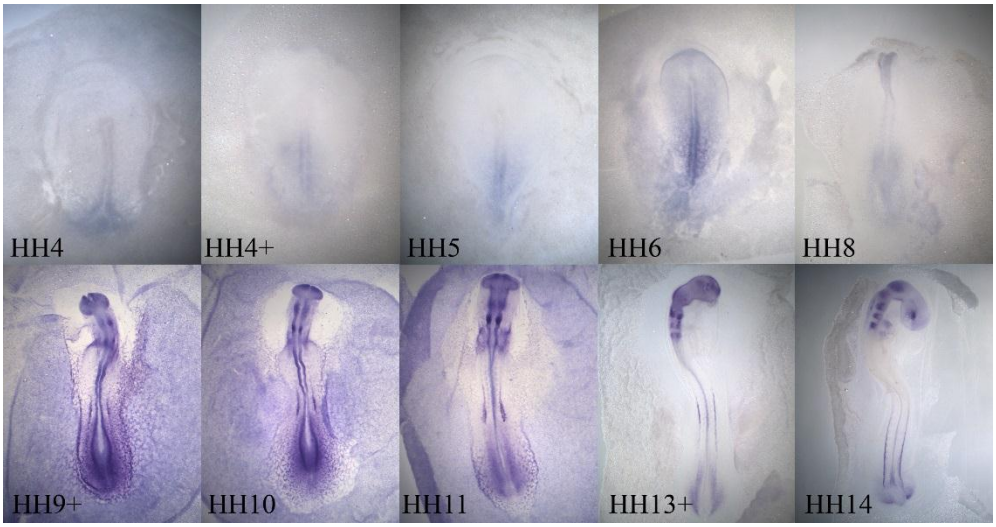


Figure 1.42 SOCS2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

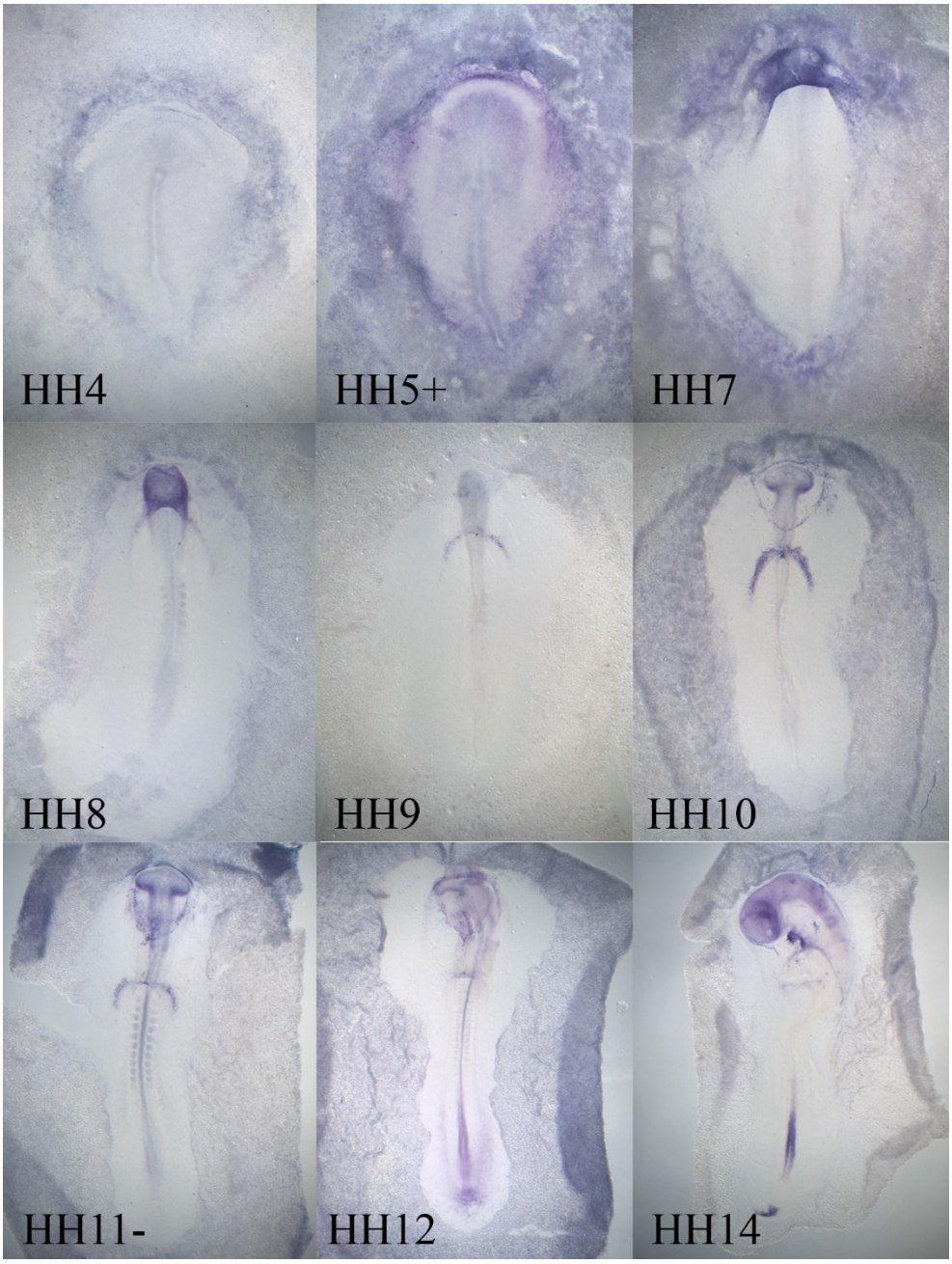


Figure 1.43 CBLN2 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view. Data kindly provided by Dr. Claire Anderson.

Figure 1.44 CXCL12 expression was studied by whole-mount *in-situ* hybridisation. Pictures were taken from the dorsal view except HH14 which was taken from ventral view.

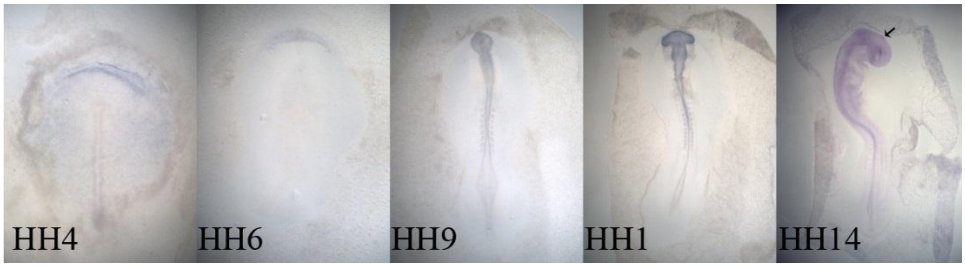


Figure 1.45 EFNA5 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

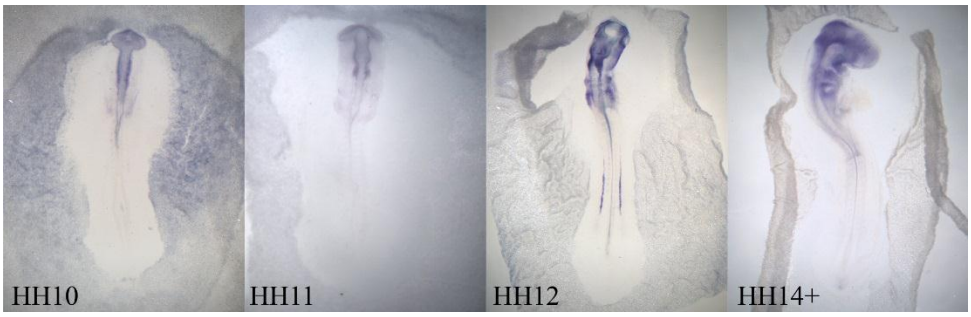


Figure 1.46 PLXNC1 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

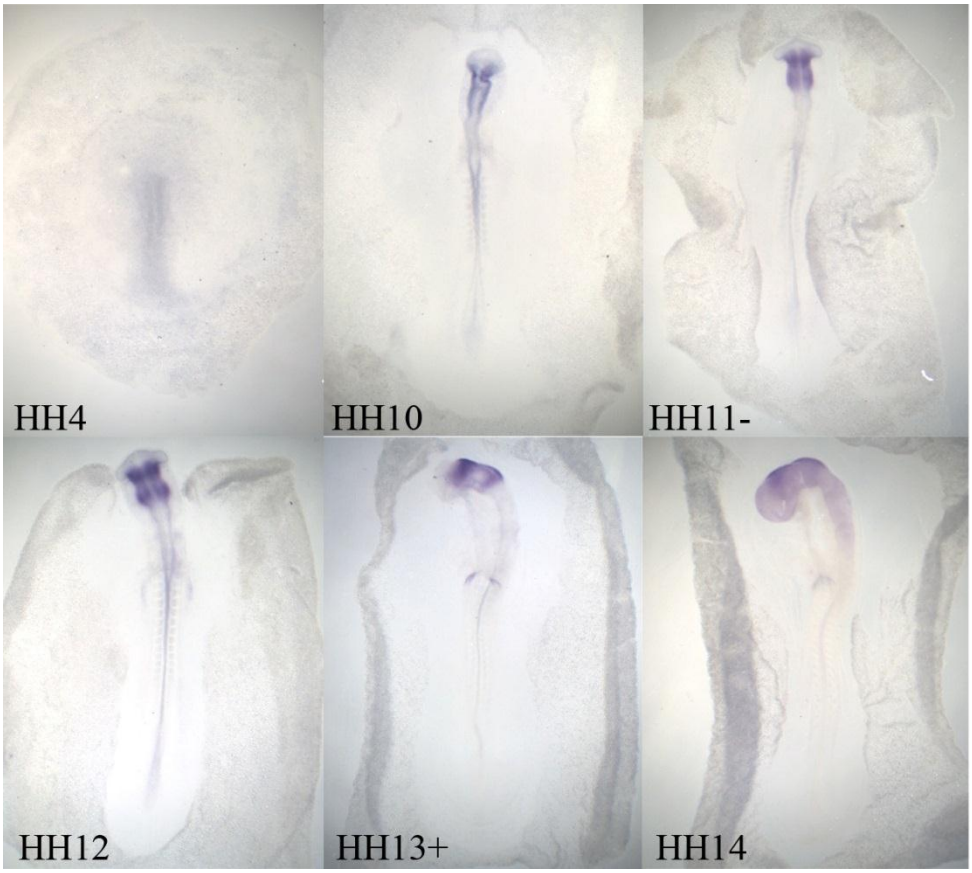


Figure 1.47 TNFRSF19 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the ventral view. Data kindly provided by Dr. Claire Anderson.

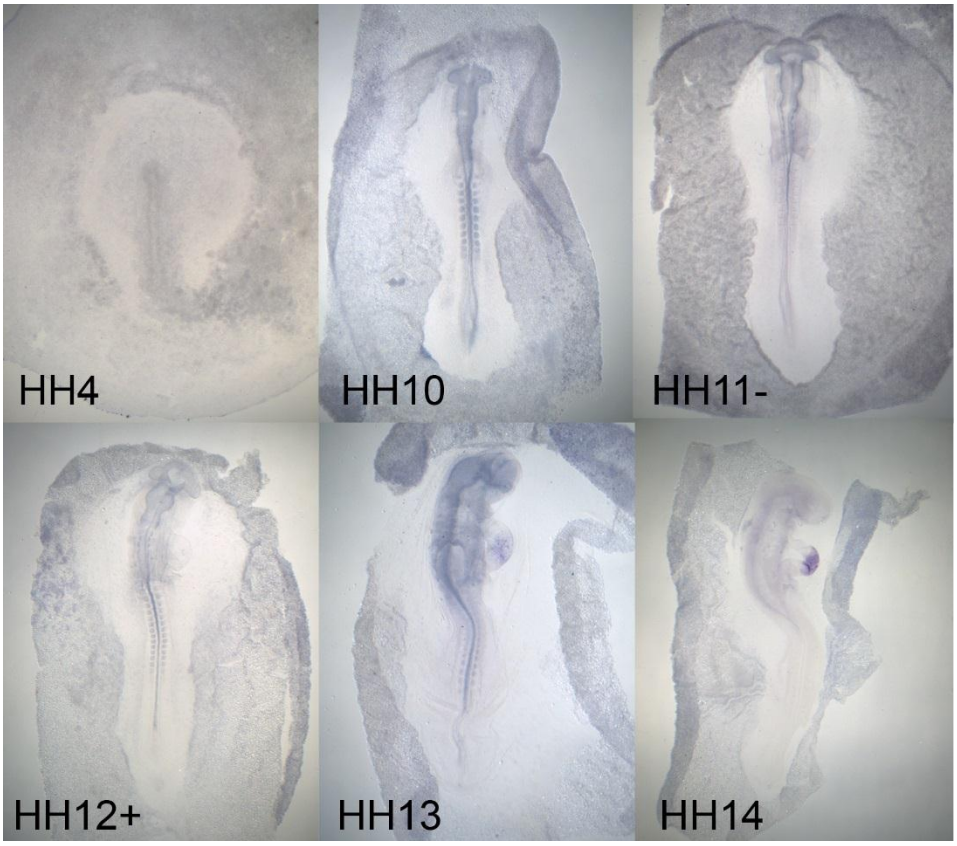
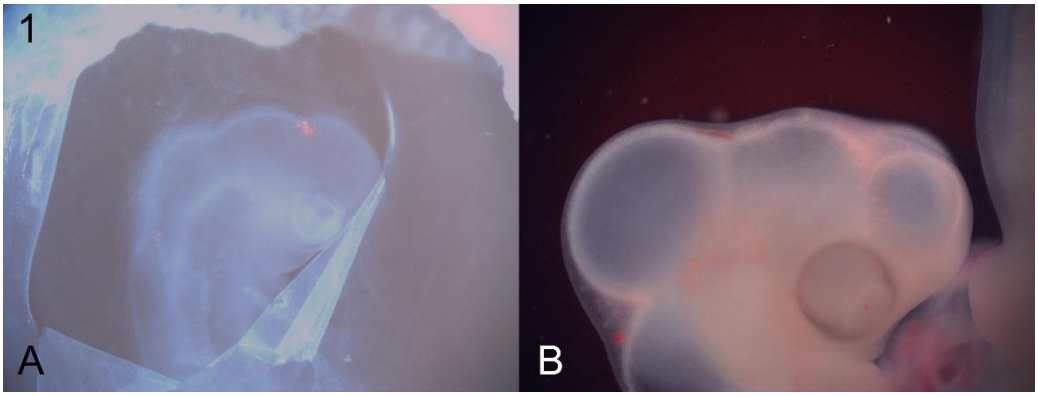
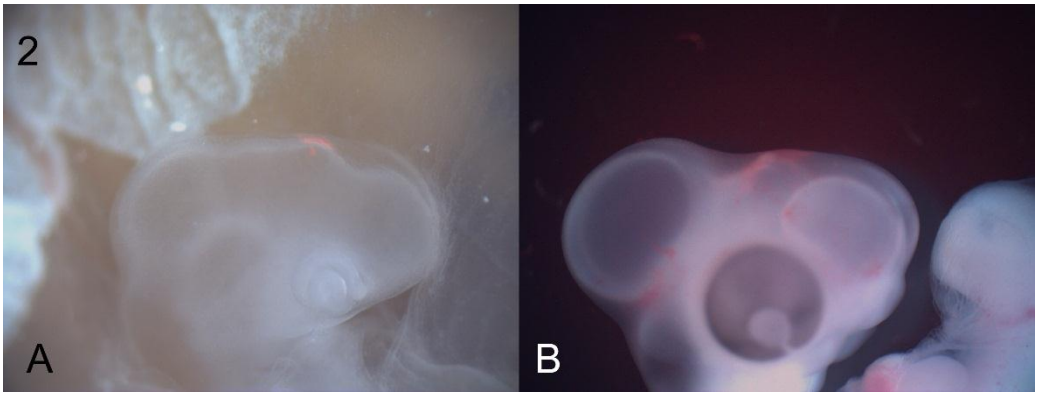


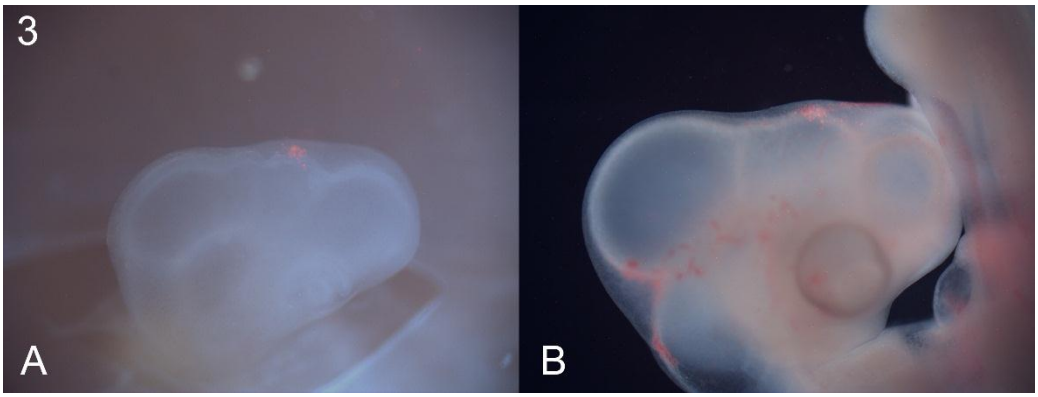
Figure 1.48 ChEST378m14 expression was studied by whole-mount *in-situ* hybridisation. All pictures were taken from the dorsal view. Data kindly provided by Dr. Claire Anderson.

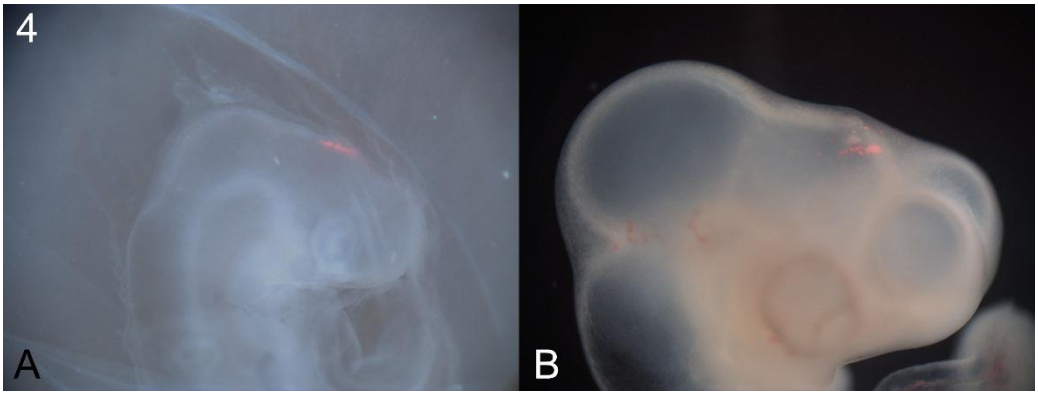
2. Fate Mapping Data

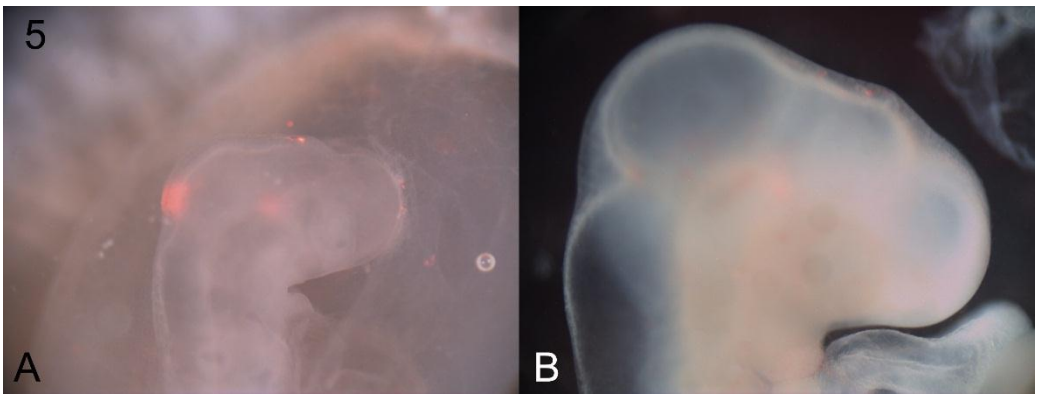
The following pictures show the fate mapping data for each embry. They are DiI labelled pictures overlapped with the raw embryo pictures. All picture A were taken before embryos were incubated (HH14) and all picture B were taken two days after incubation (~ HH22).

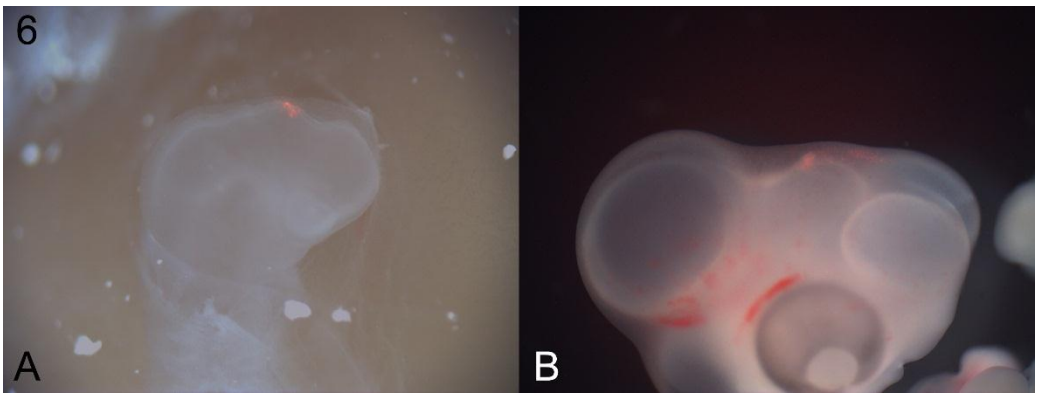


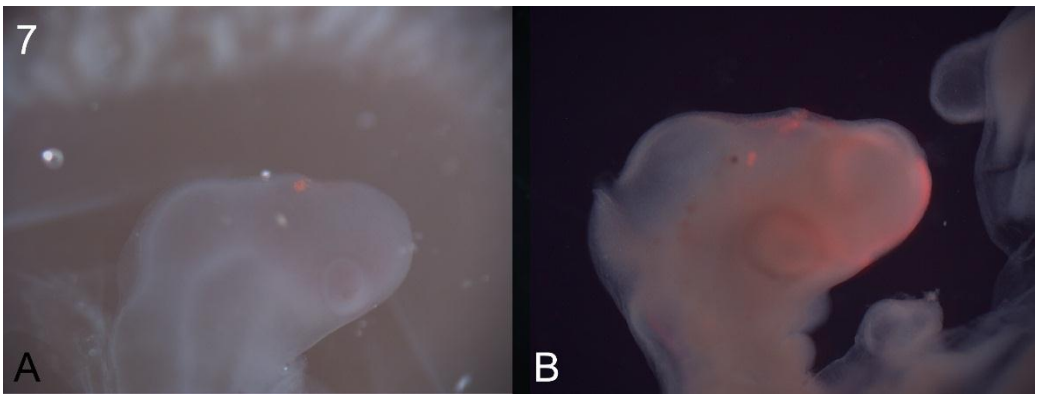


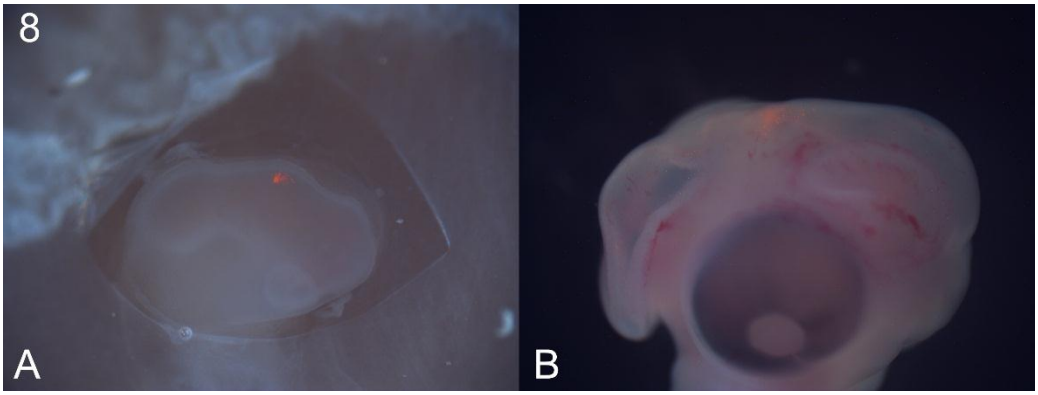


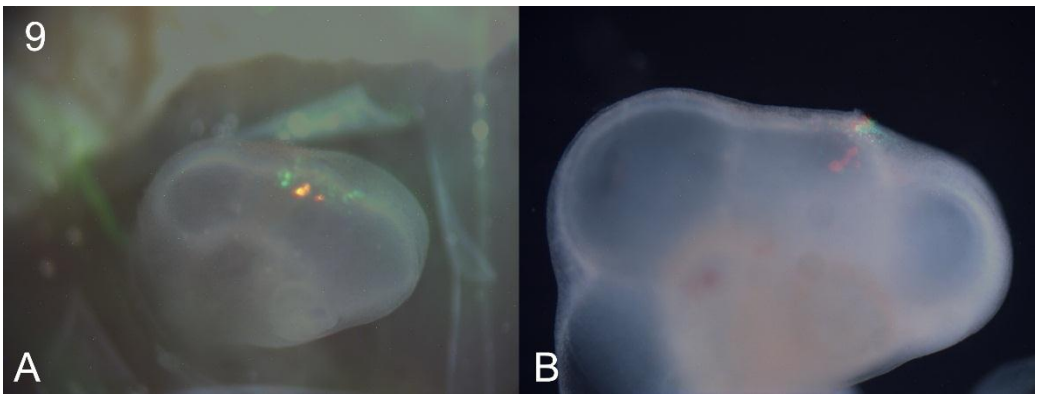

4
A
B

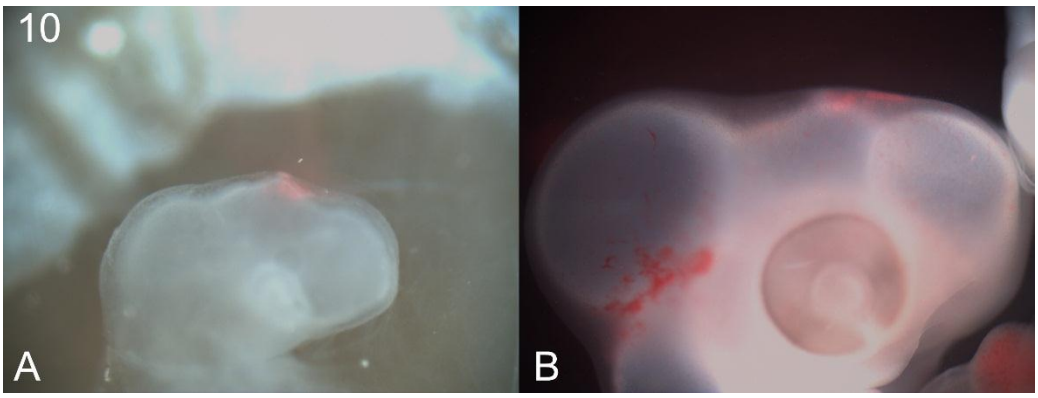

5
A
B

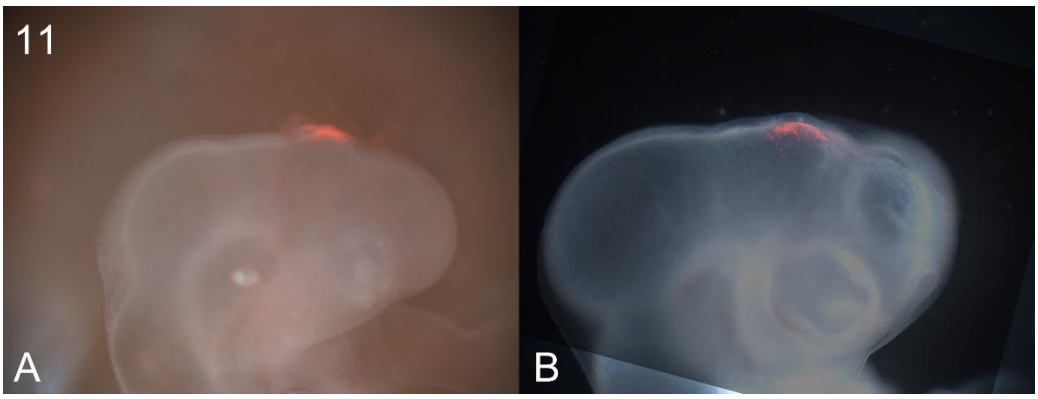

6
A
B

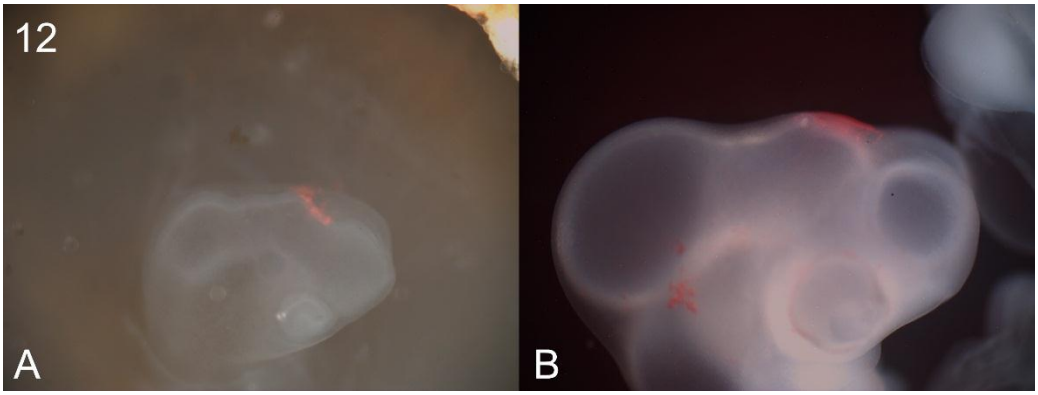

7
A
B

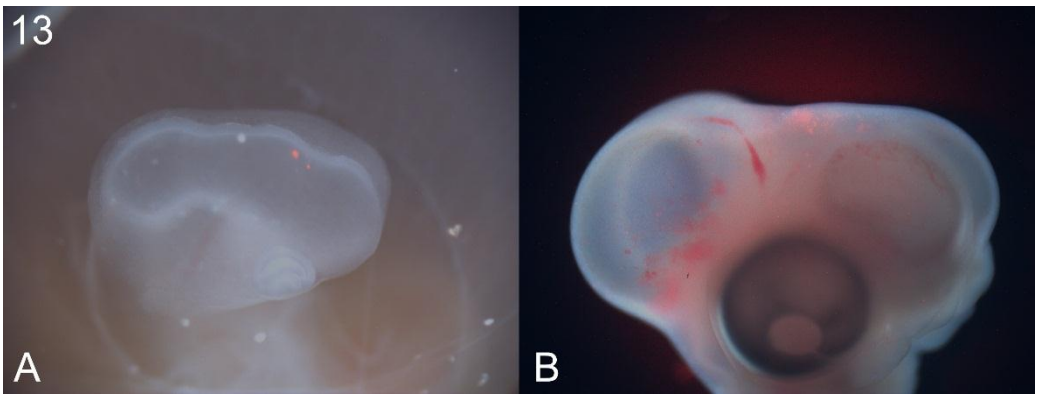

8
A
B

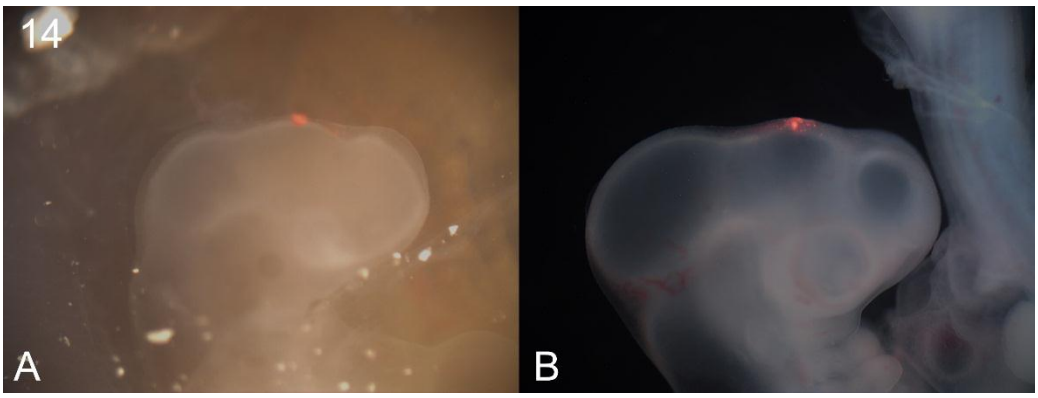

9
A
B

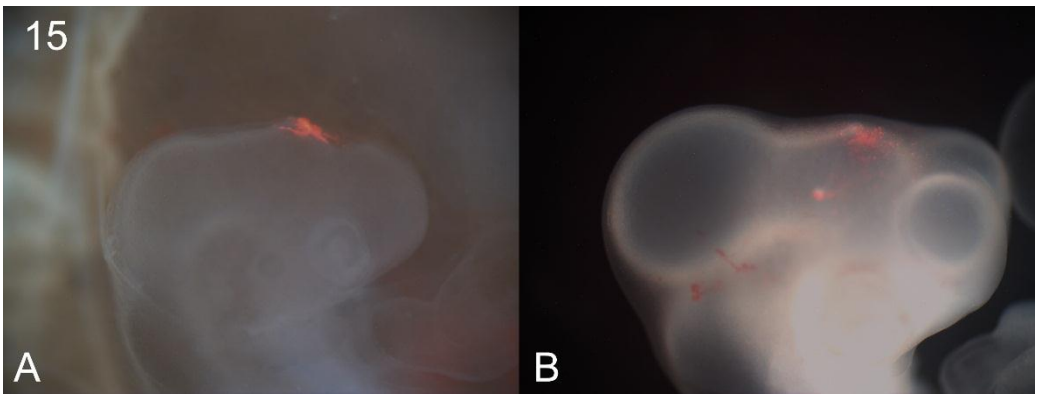

10
A
B

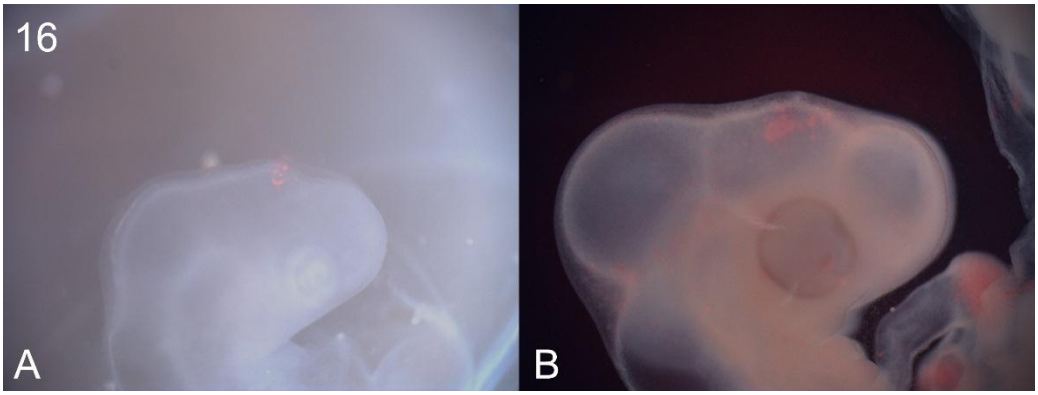

11
A
B

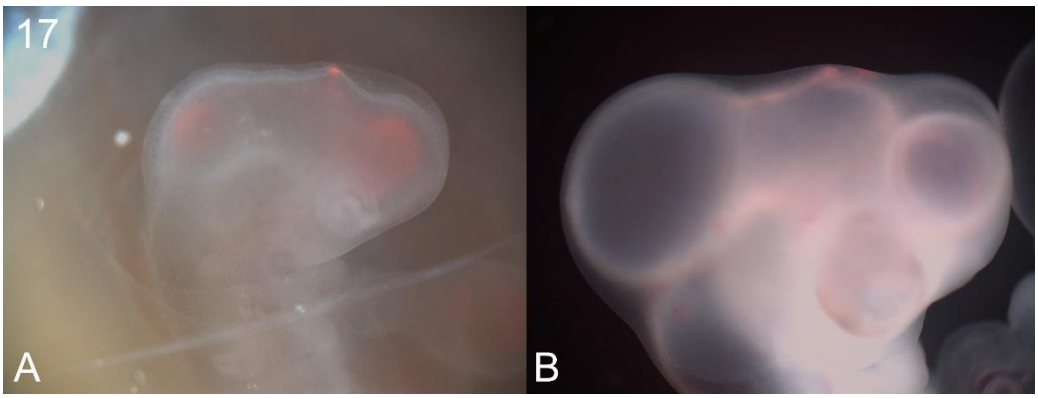

12
A
B

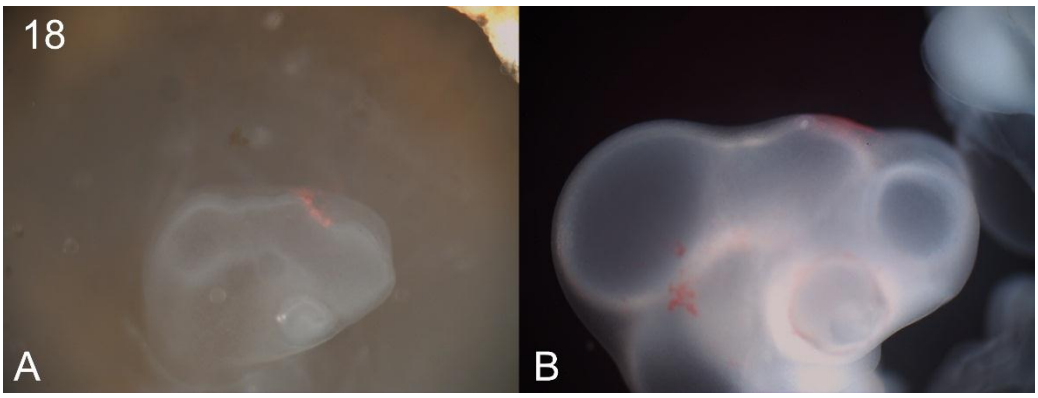

13
A
B

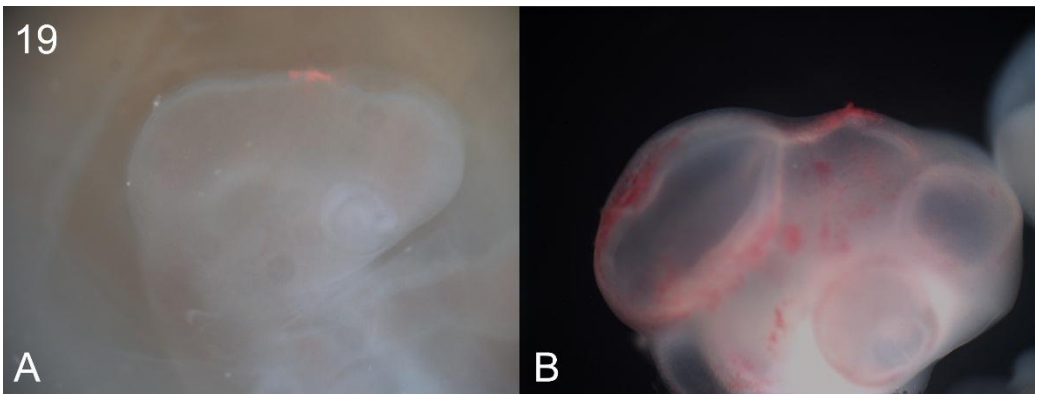

14
A
B

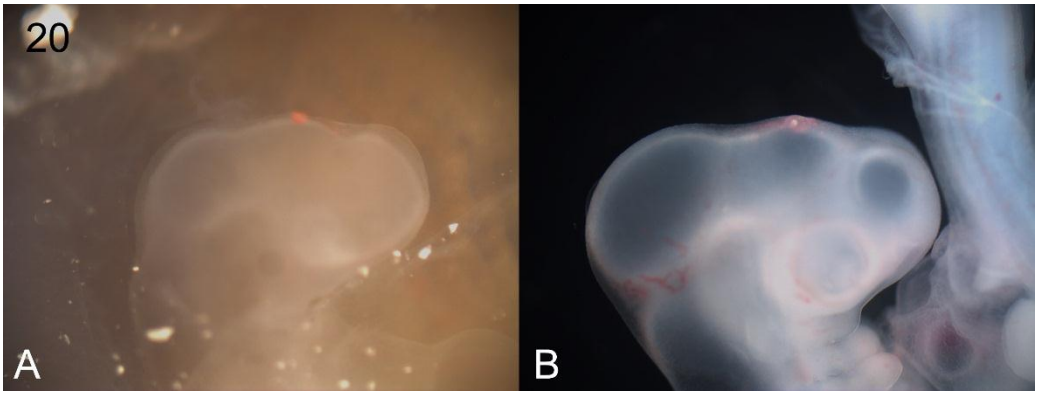

15
A
B

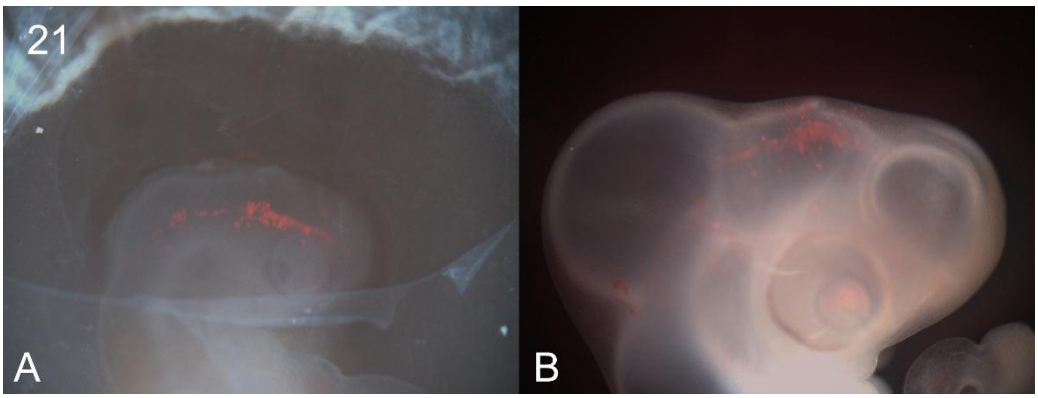

16
A
B

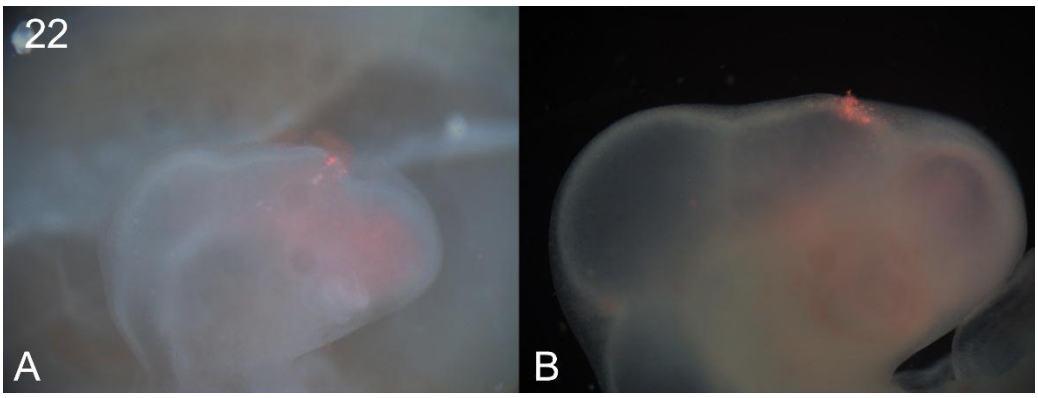

17
A
B

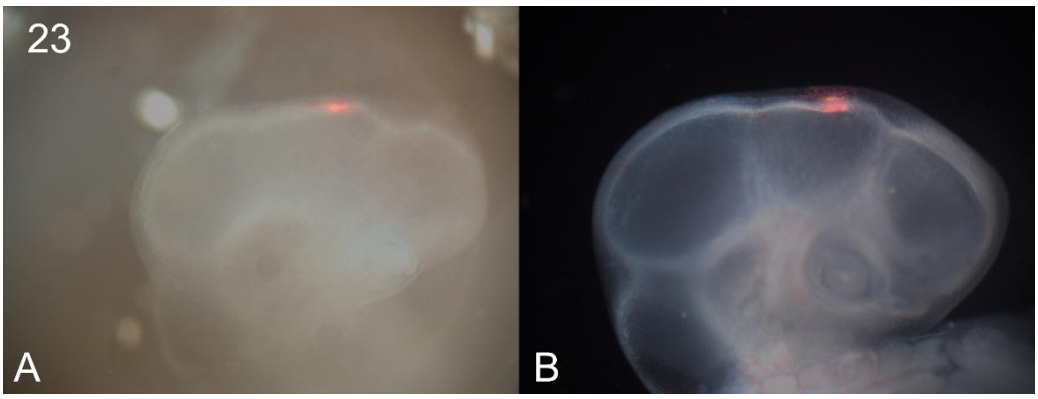

18
A
B

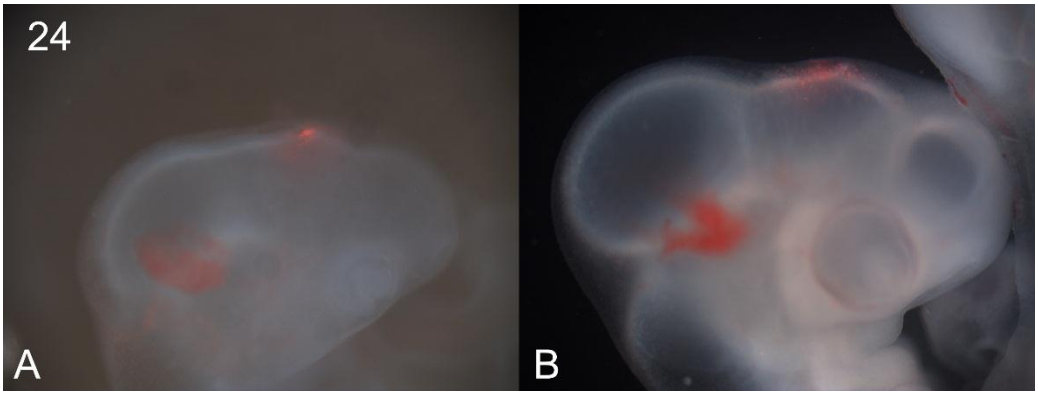

19
A
B

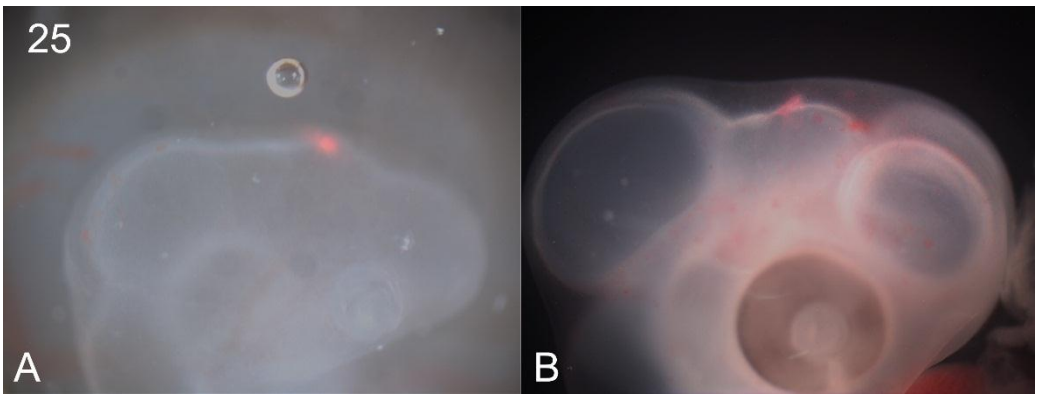

20
A
B

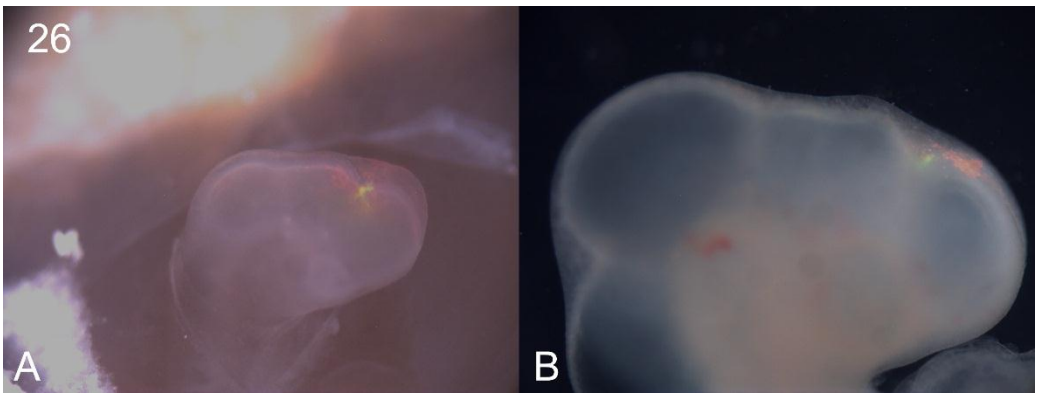

21
A
B

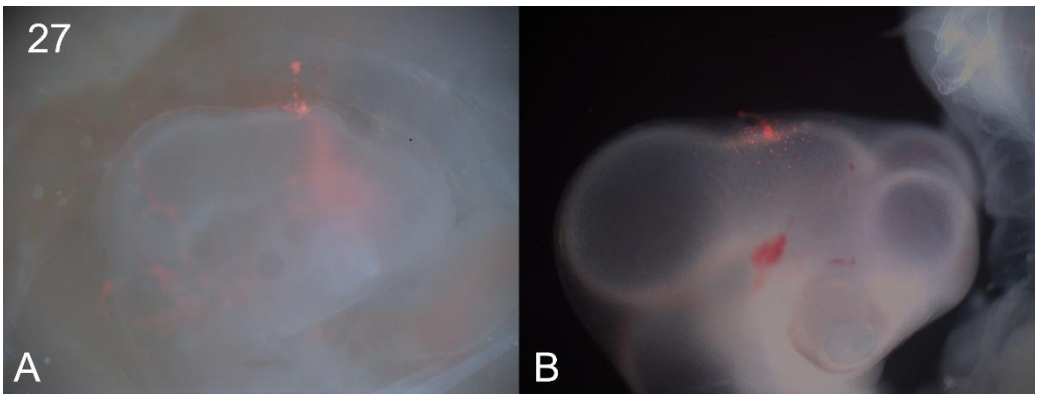

22
A
B

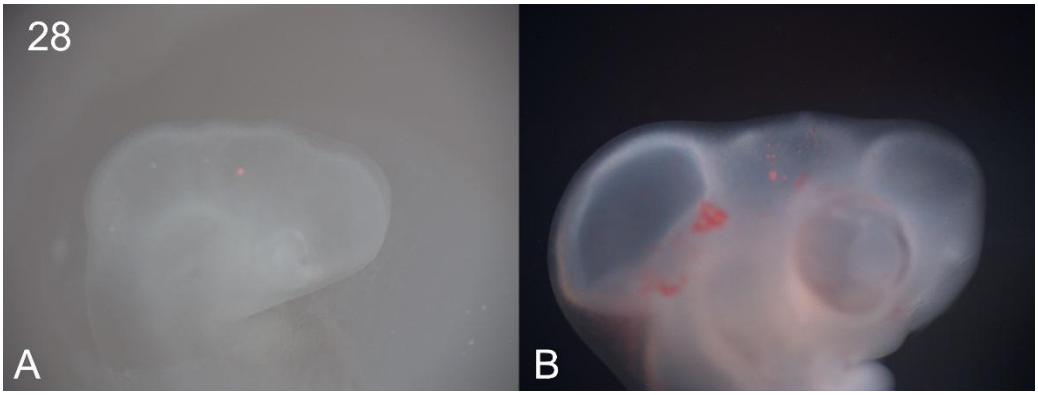

23
A
B

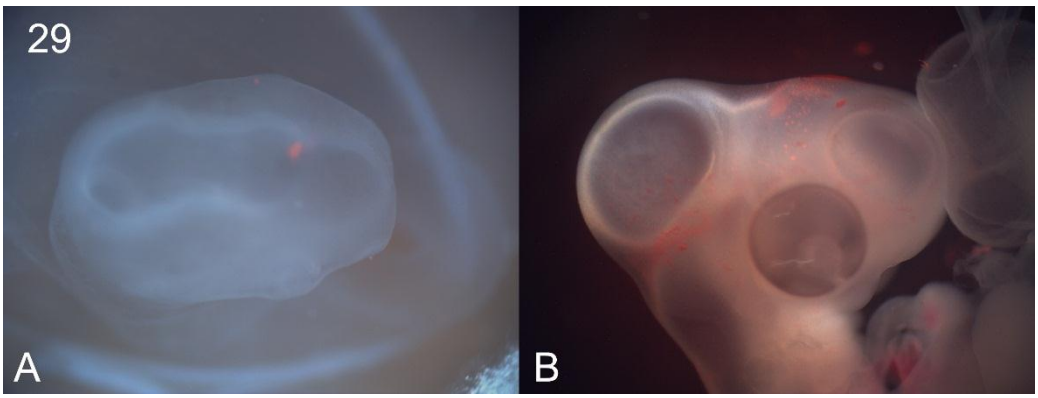

24
A
B

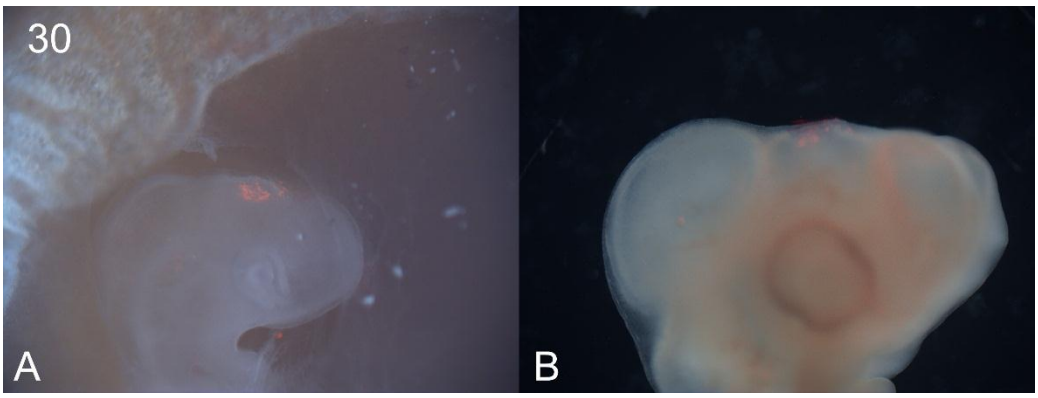

25
A
B

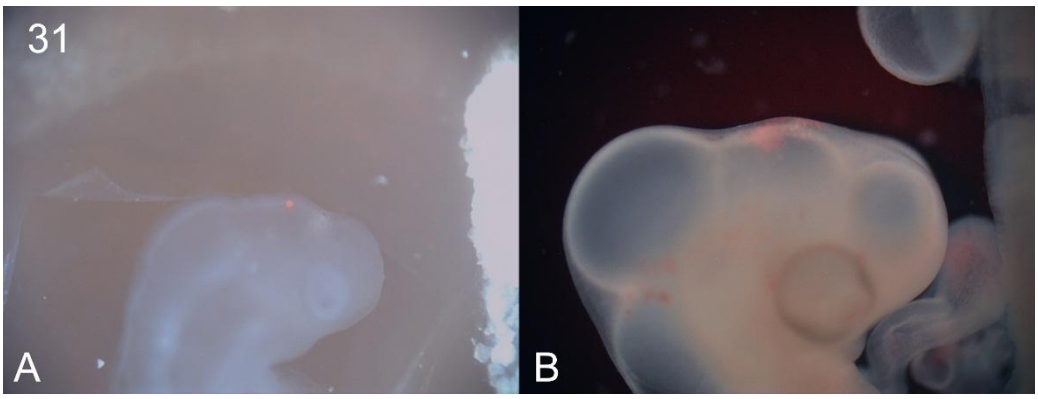

26
A
B

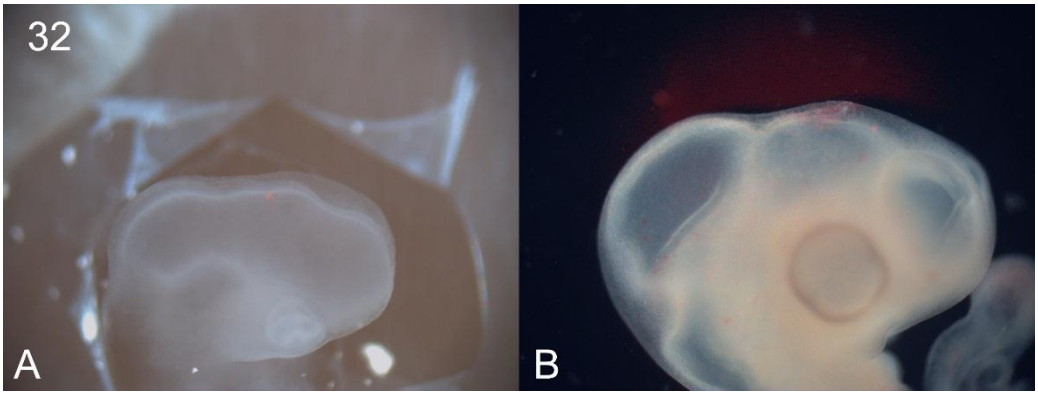

27
A
B

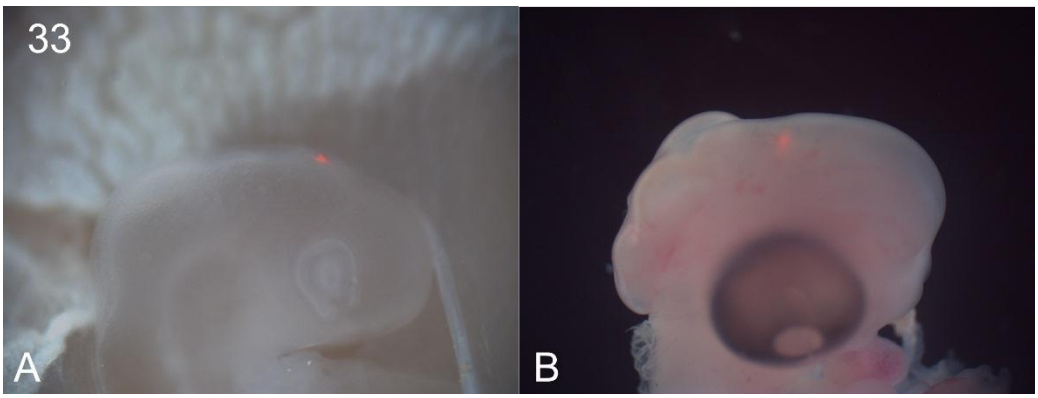

28
A
B

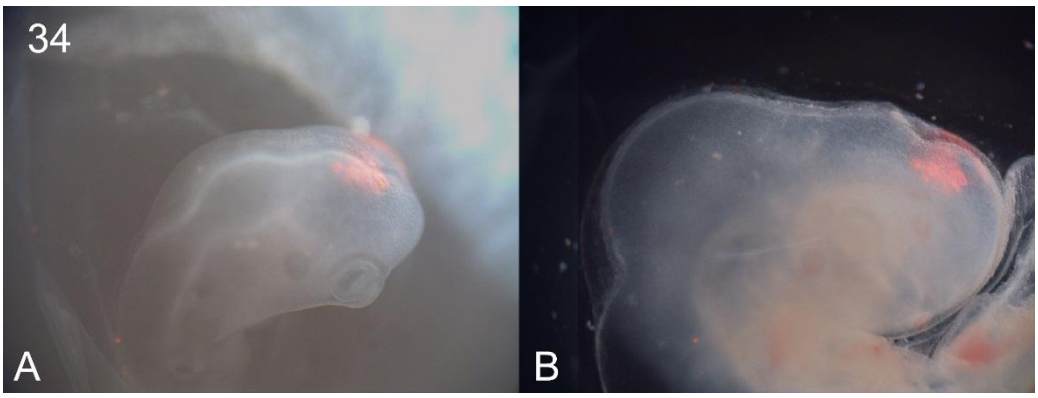

29
A
B

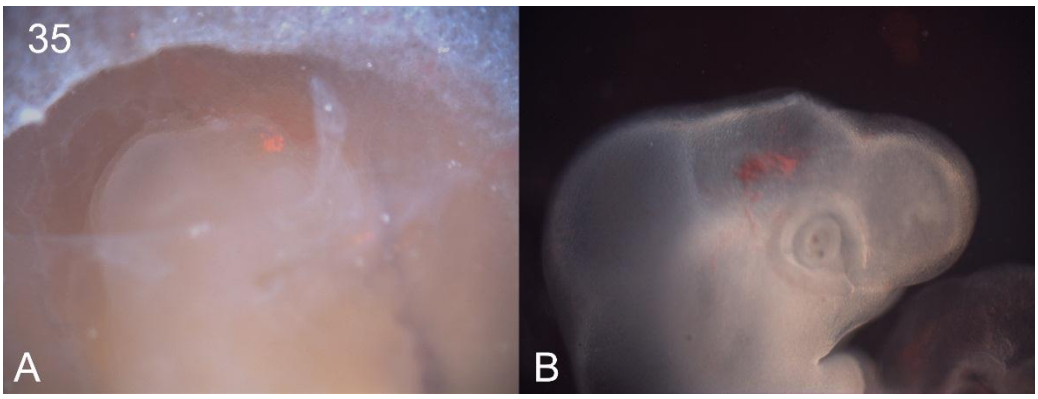

30
A
B

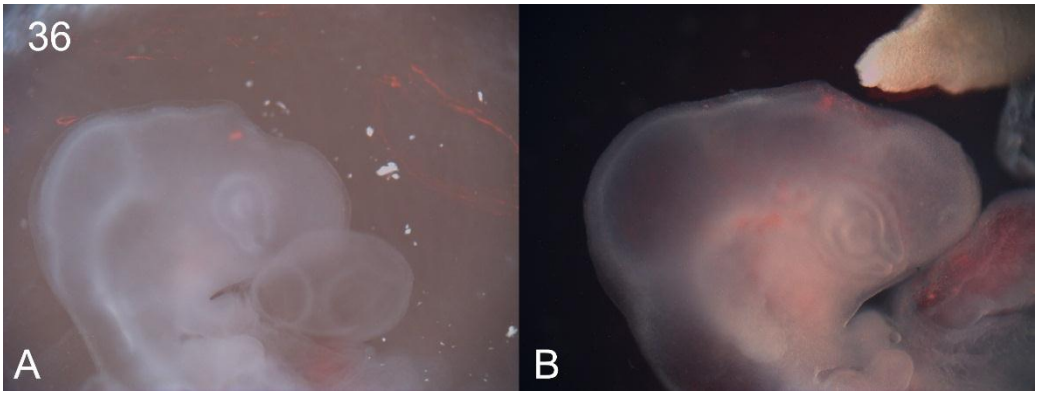

31
A
B

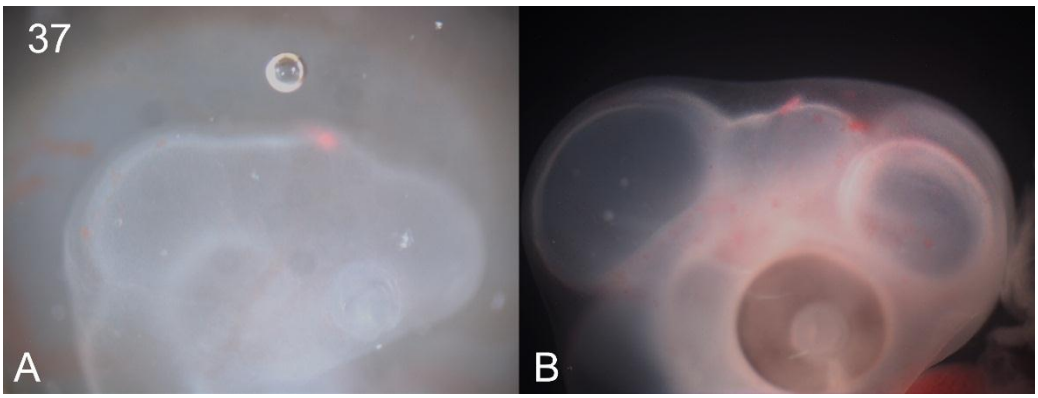

32
A
B

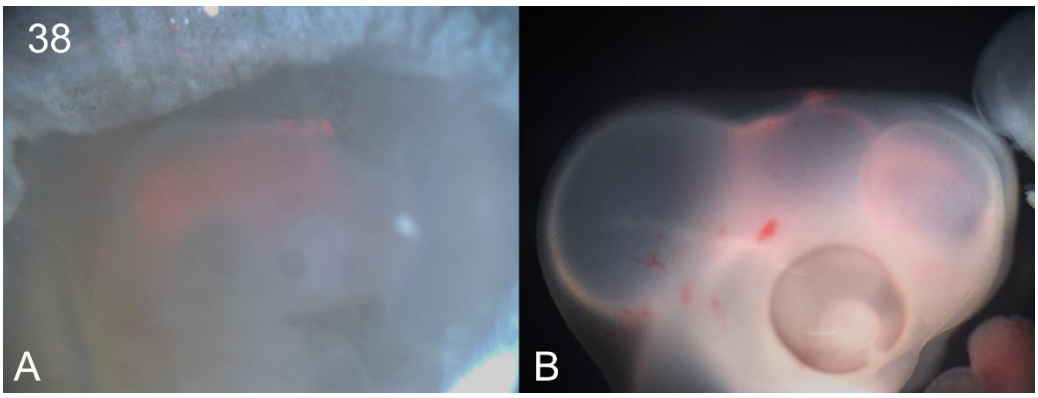

33
A
B

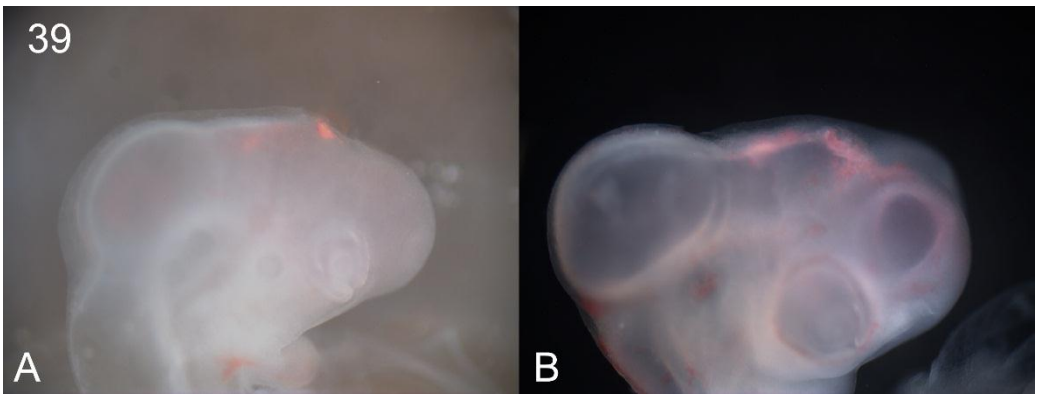

34
A
B

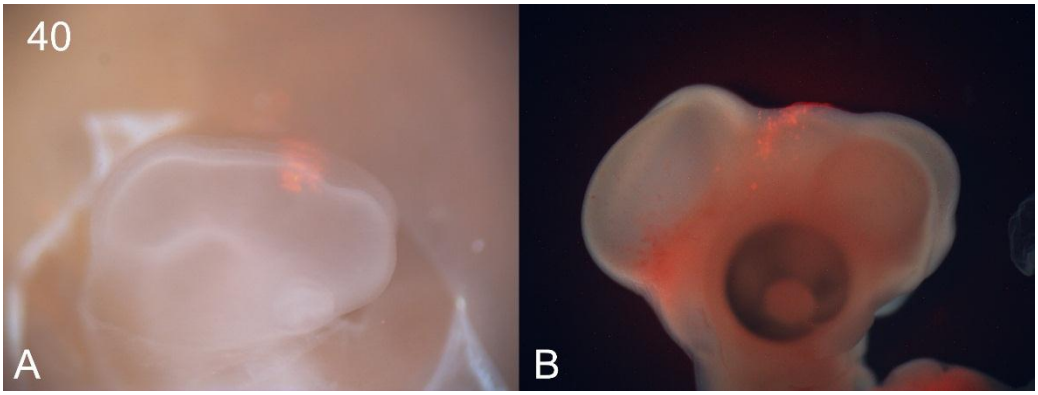

35
A
B

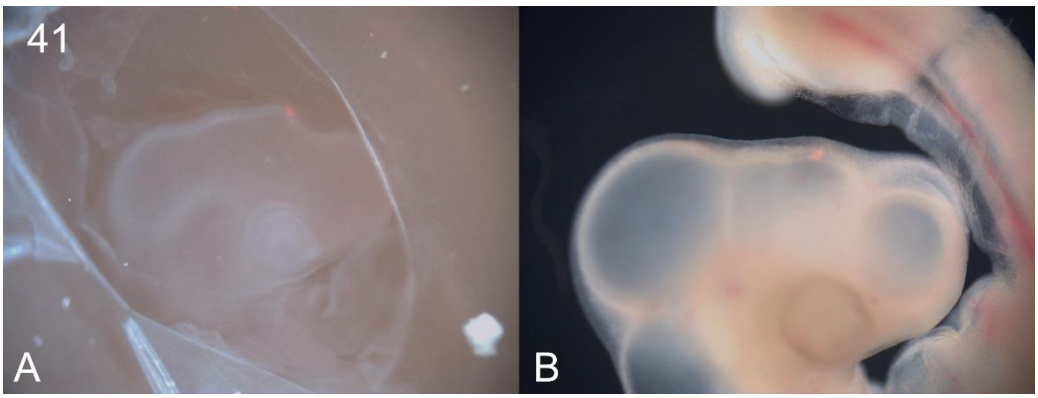

36
A
B

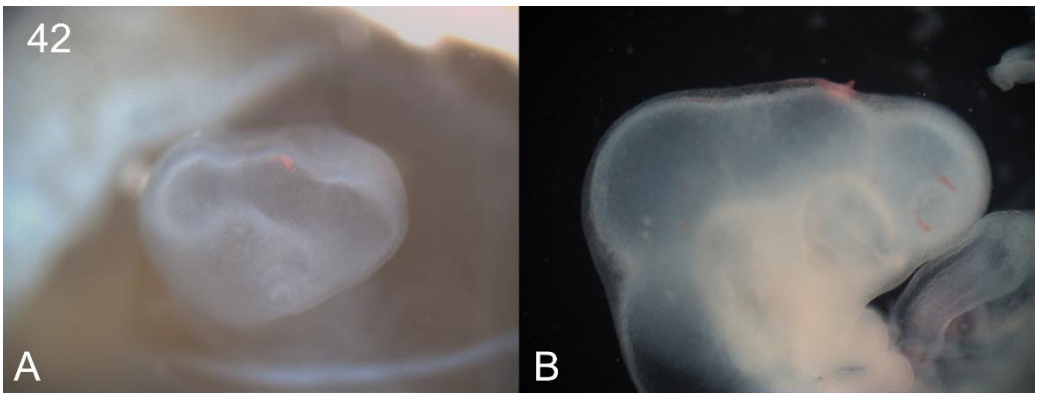

37
A
B

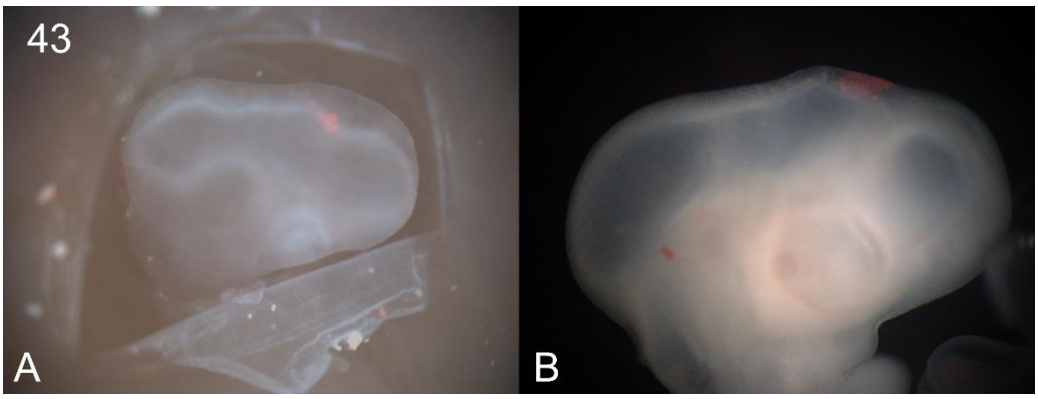

38
A
B

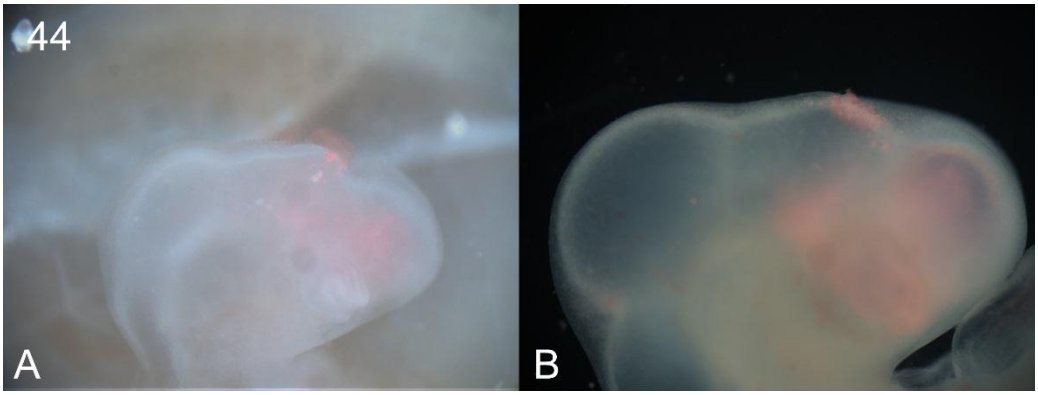

39
A
B

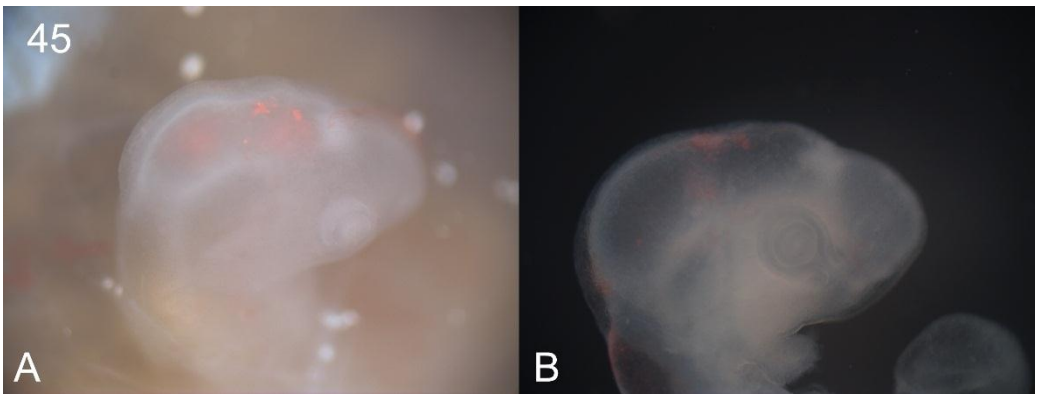

40
A
B

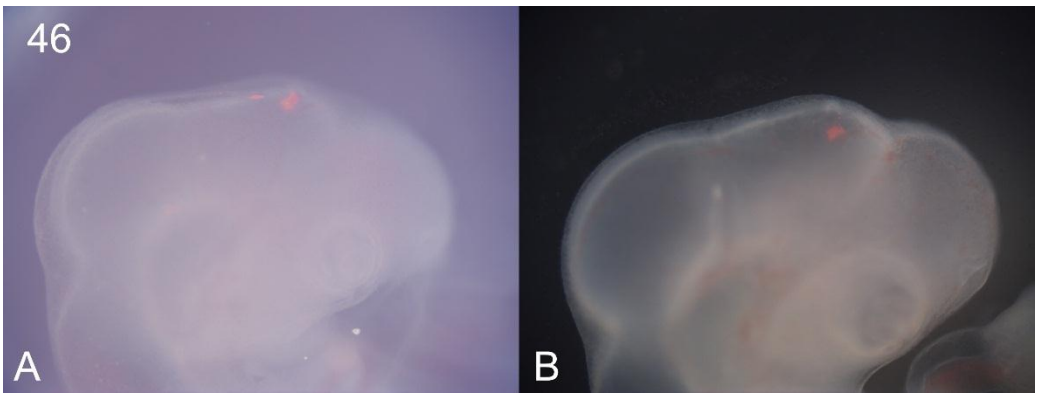

41
A
B

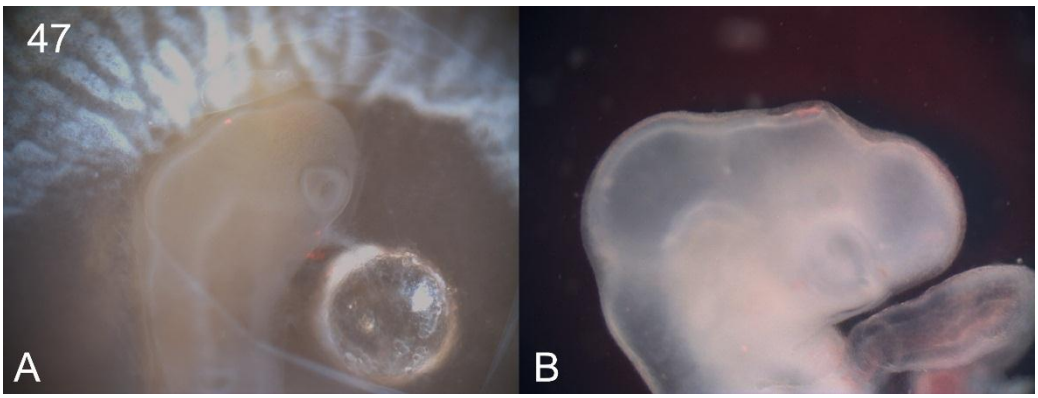

42
A
B

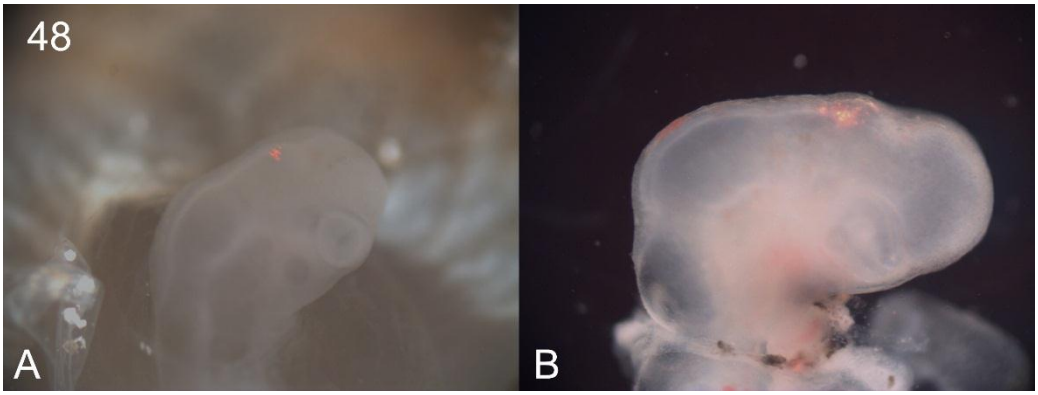

43
A
B

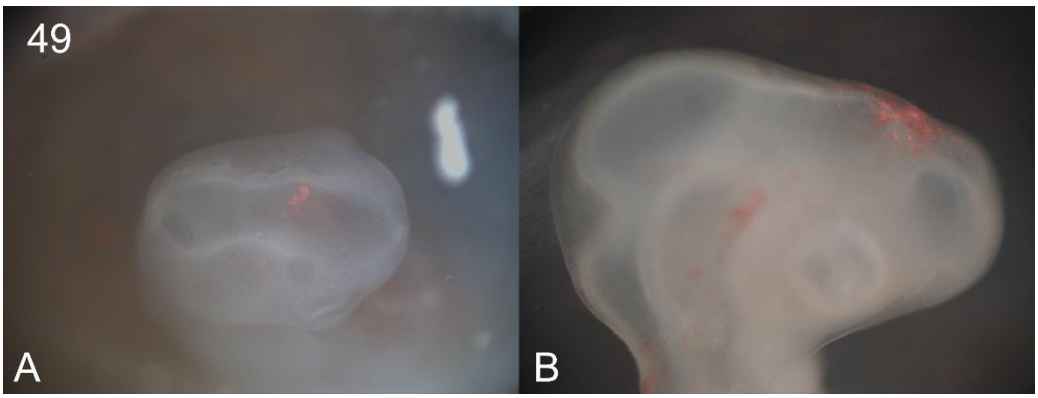

44
A
B

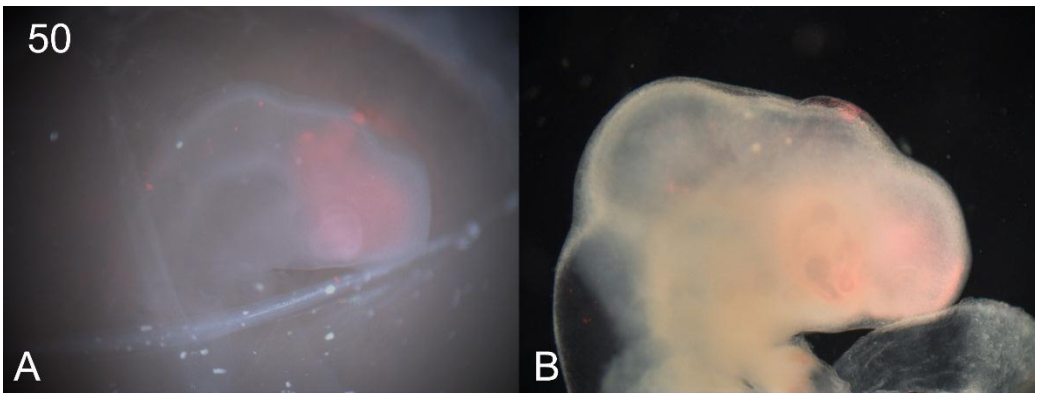

45
A
B

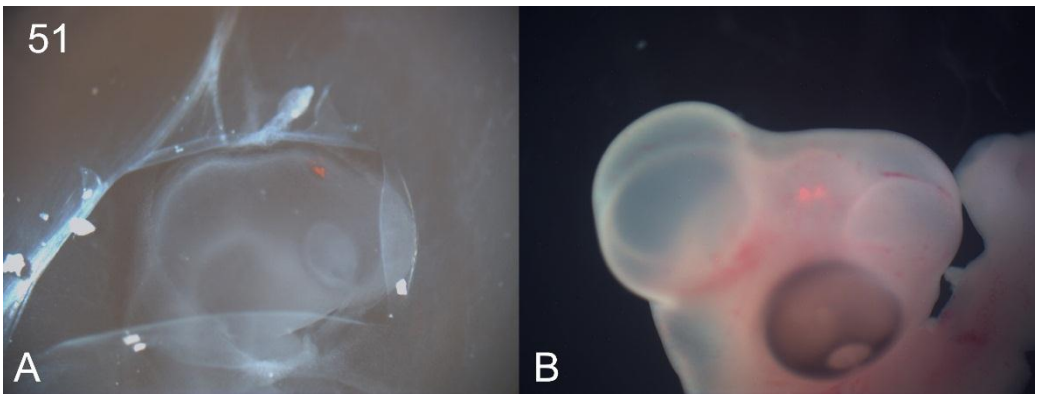

46
A
B

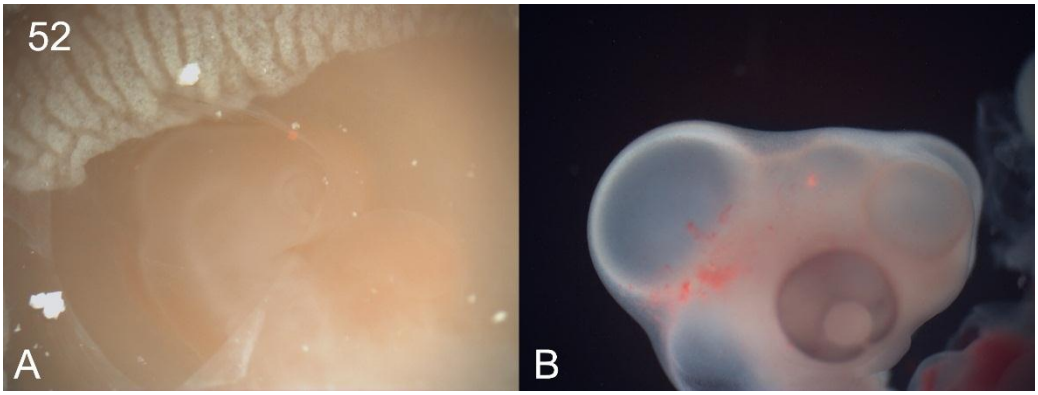

47
A
B

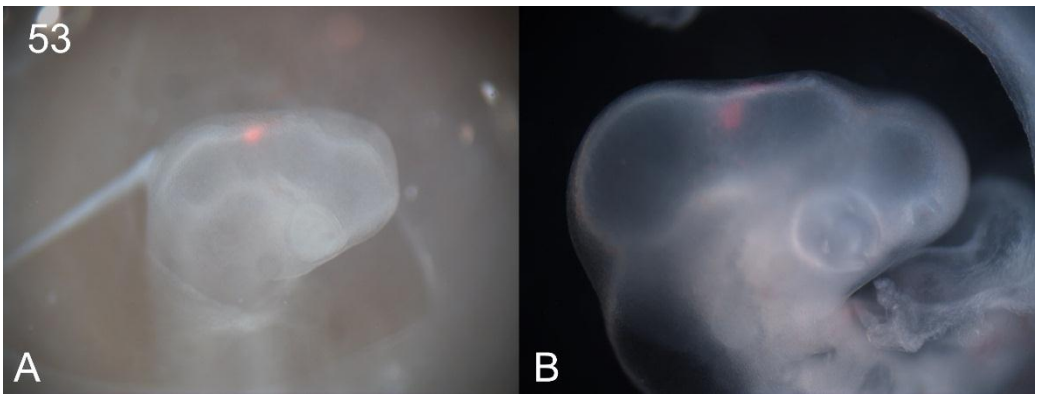

48
A
B

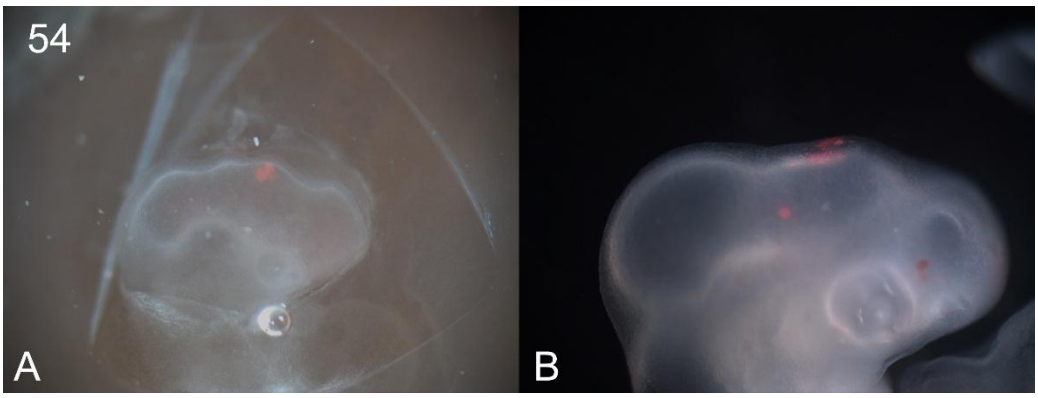

49
A
B

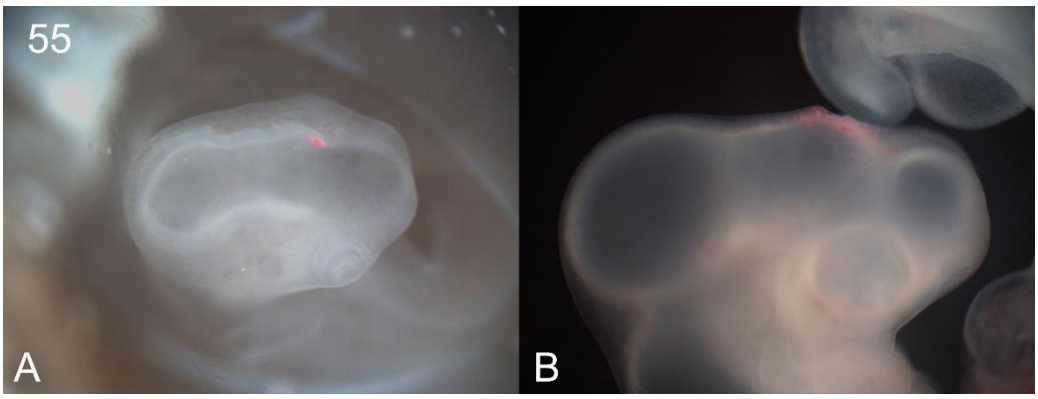

50
A
B

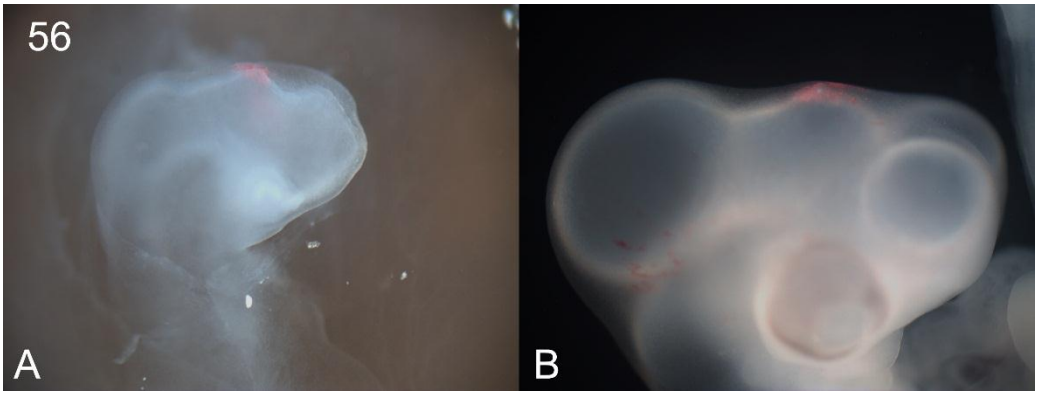

51
A
B

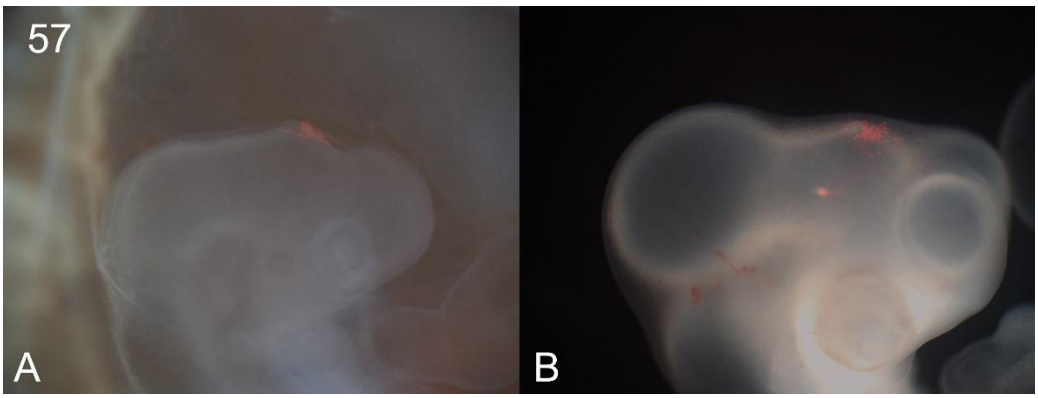

52
A
B

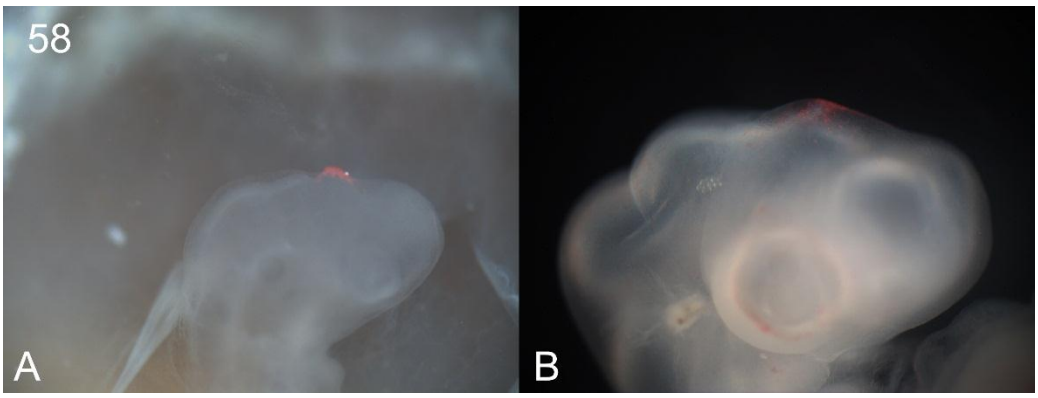

53
A
B

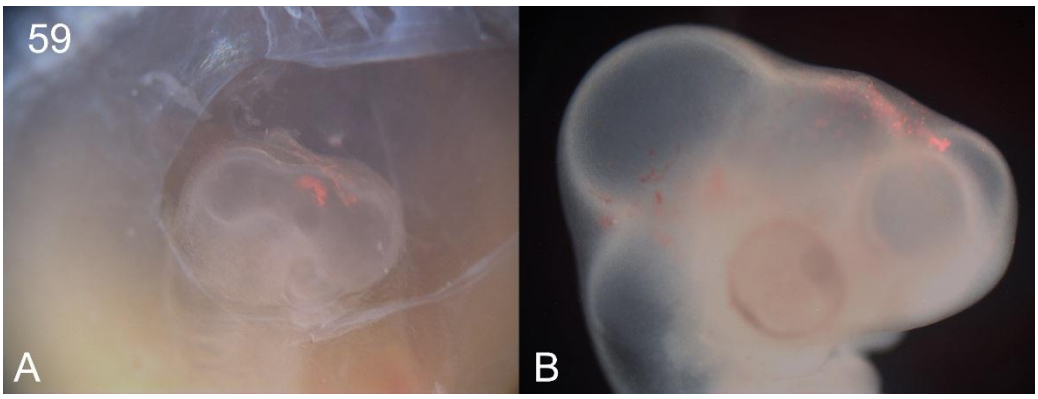

54
A
B

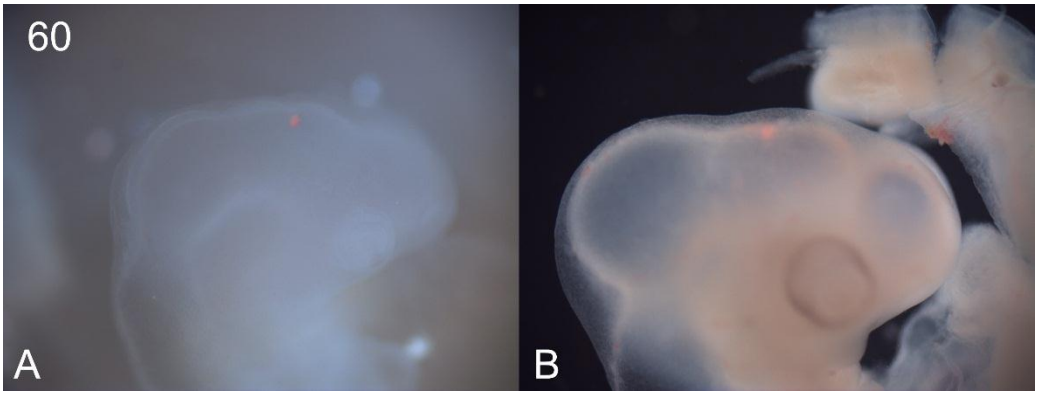

55
A
B

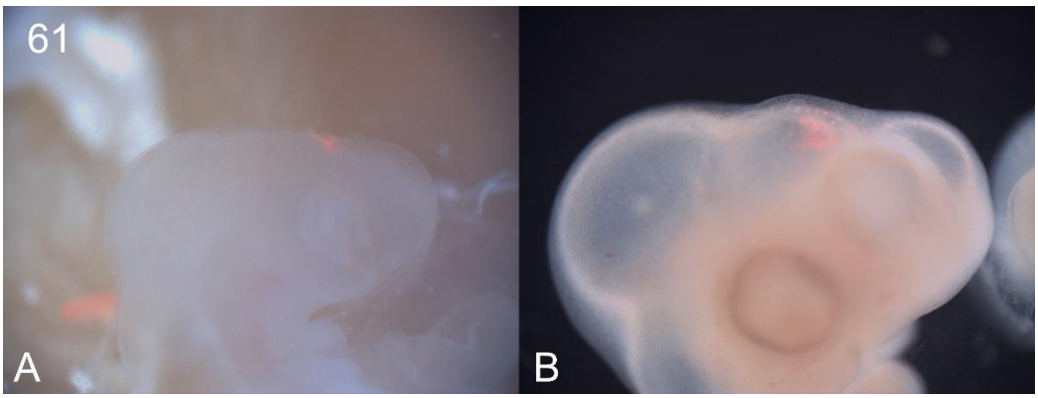

56
A
B

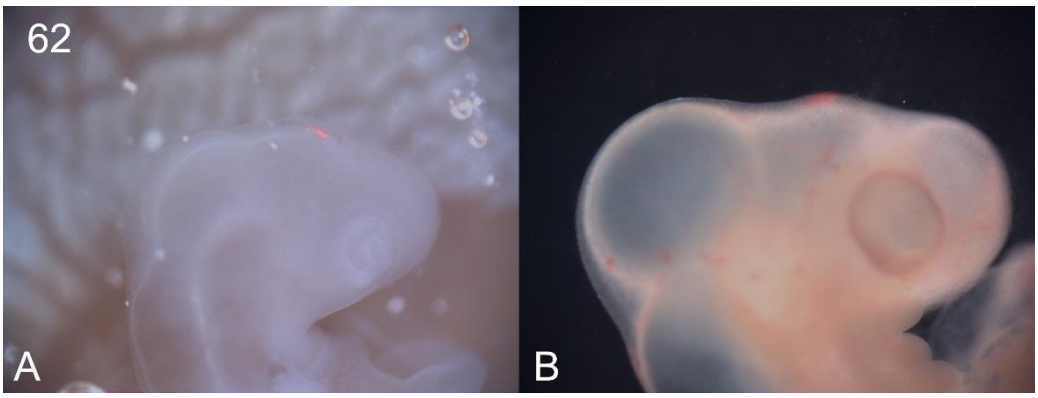

57
A
B

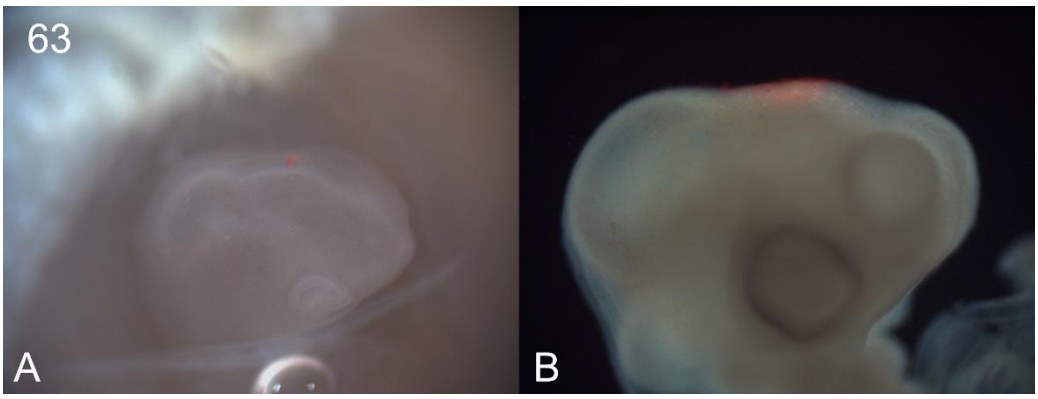

58
A
B

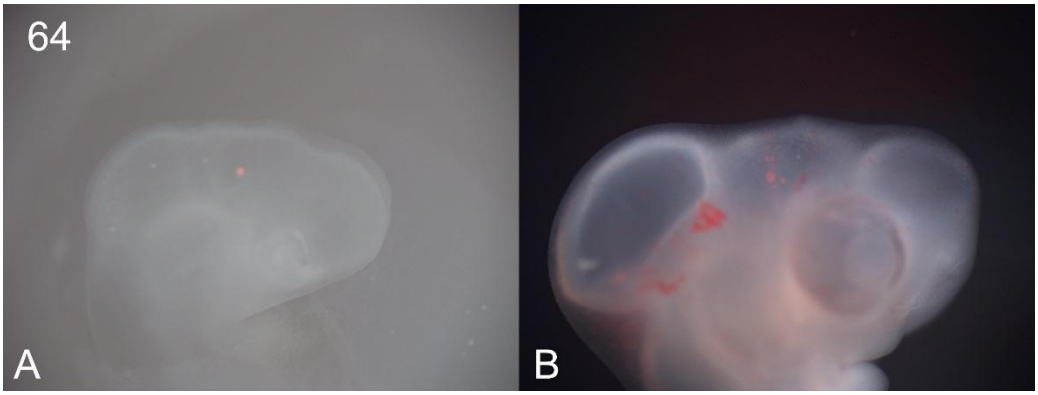

59
A
B

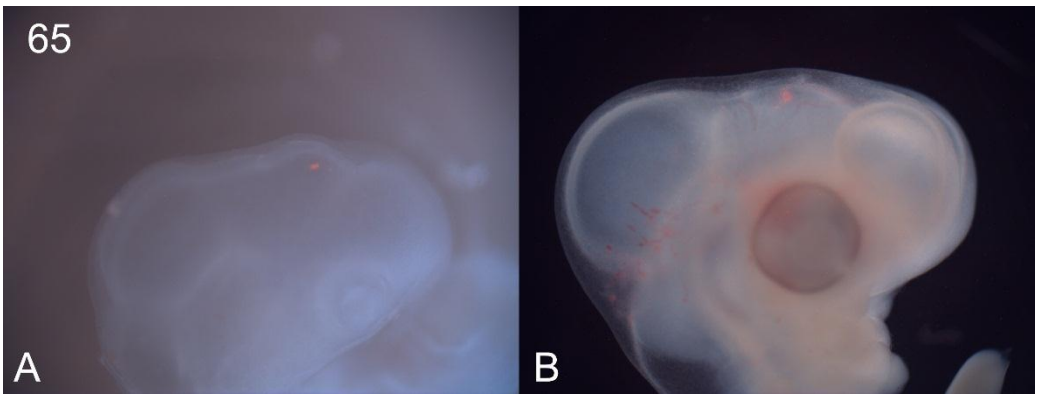

60
A
B

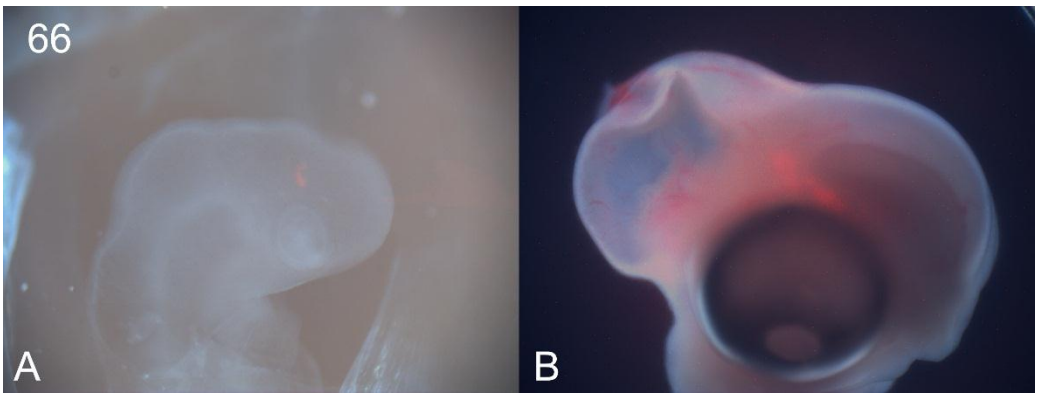

61
A
B

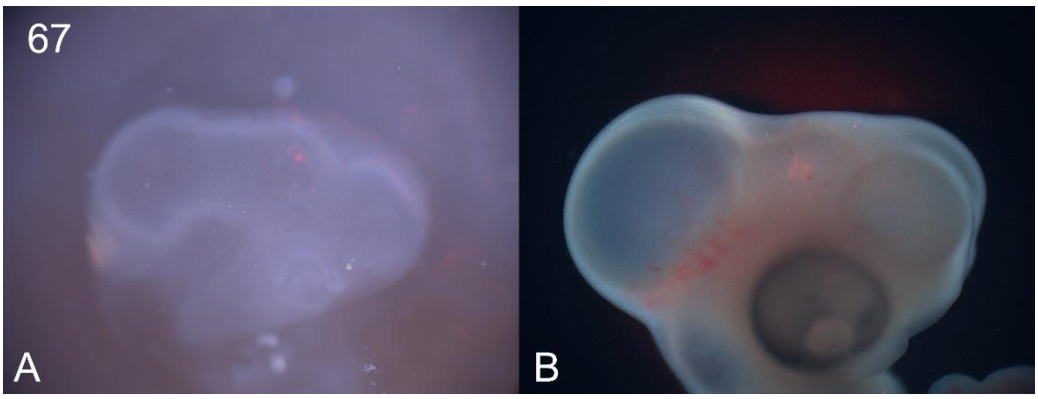

62
A
B

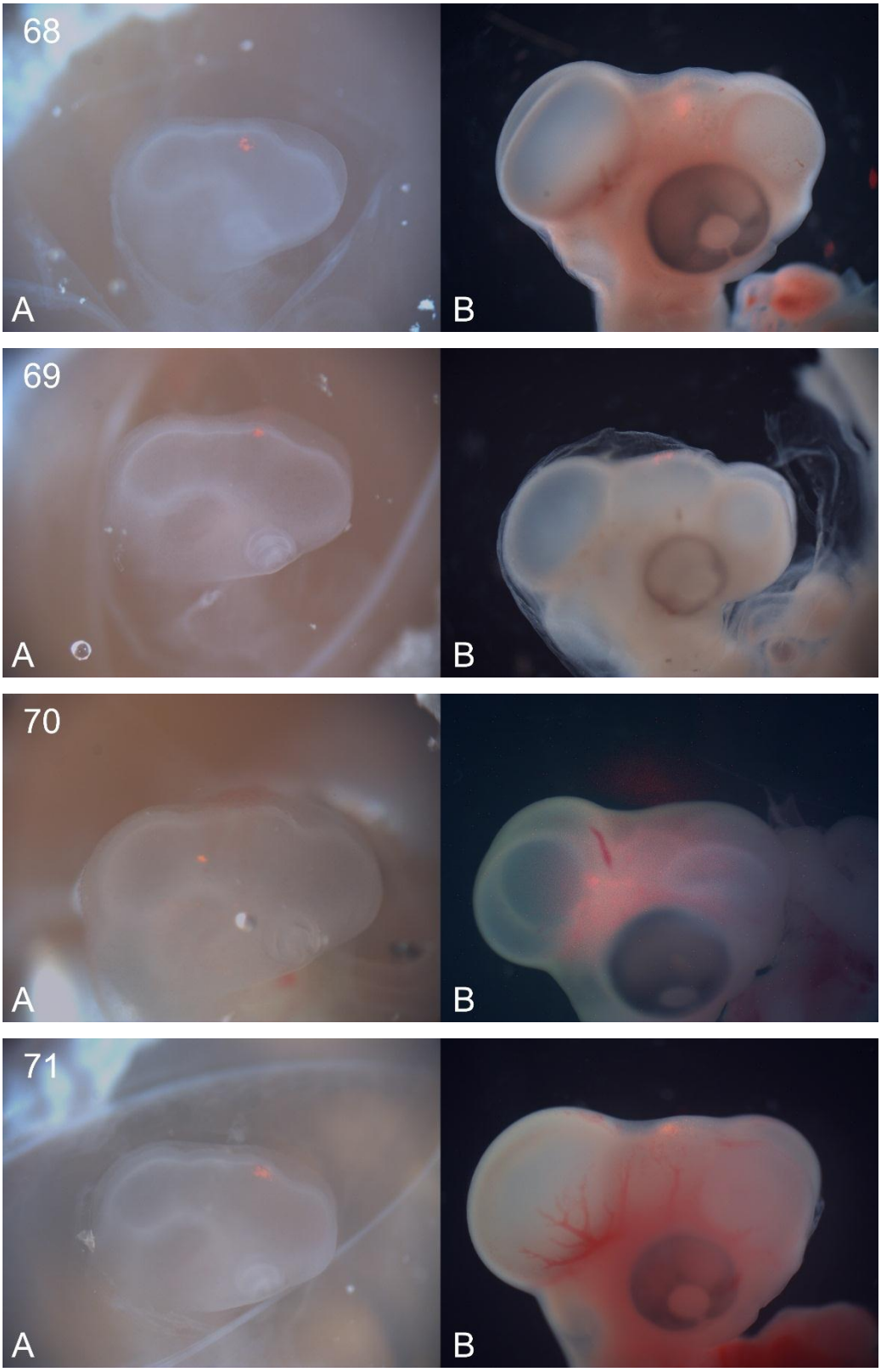

63
A
B

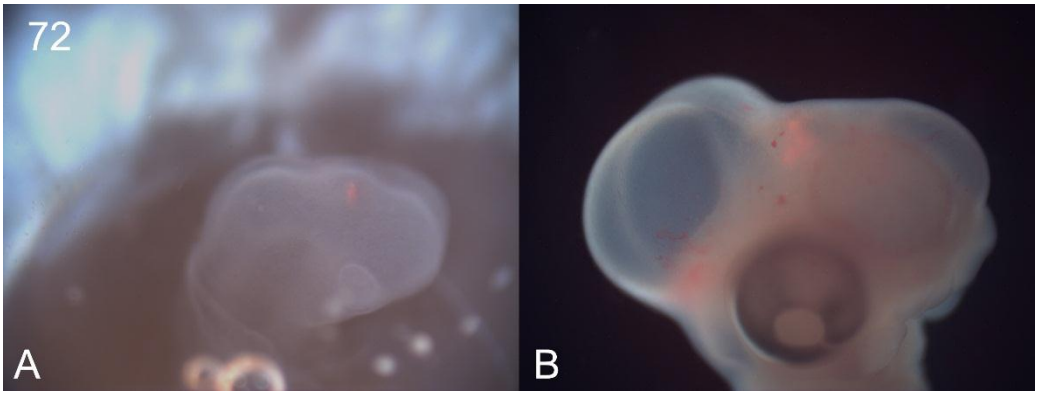

64
A
B

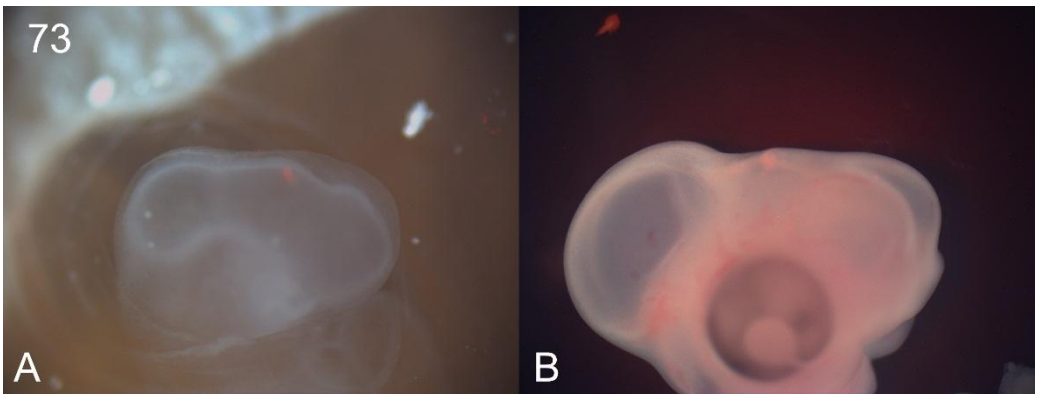

65
A
B

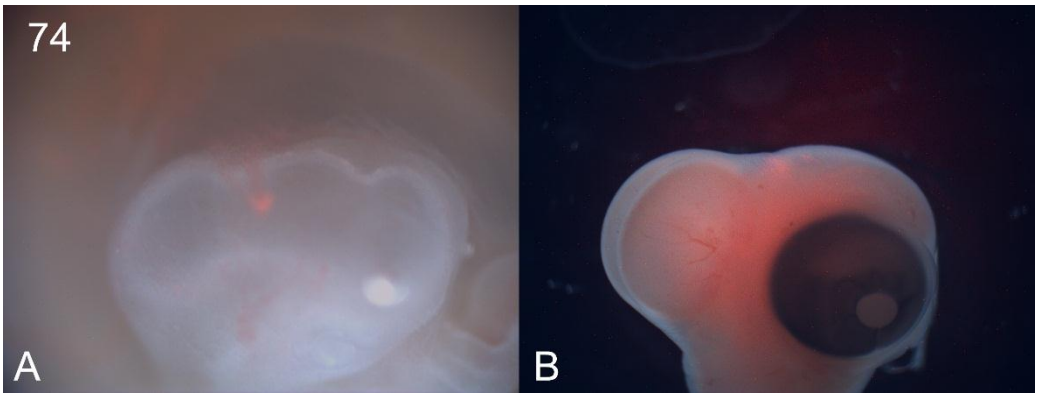

66
A
B


67
A
B


68
A
B
69
A
B
70
A
B
71
A
B

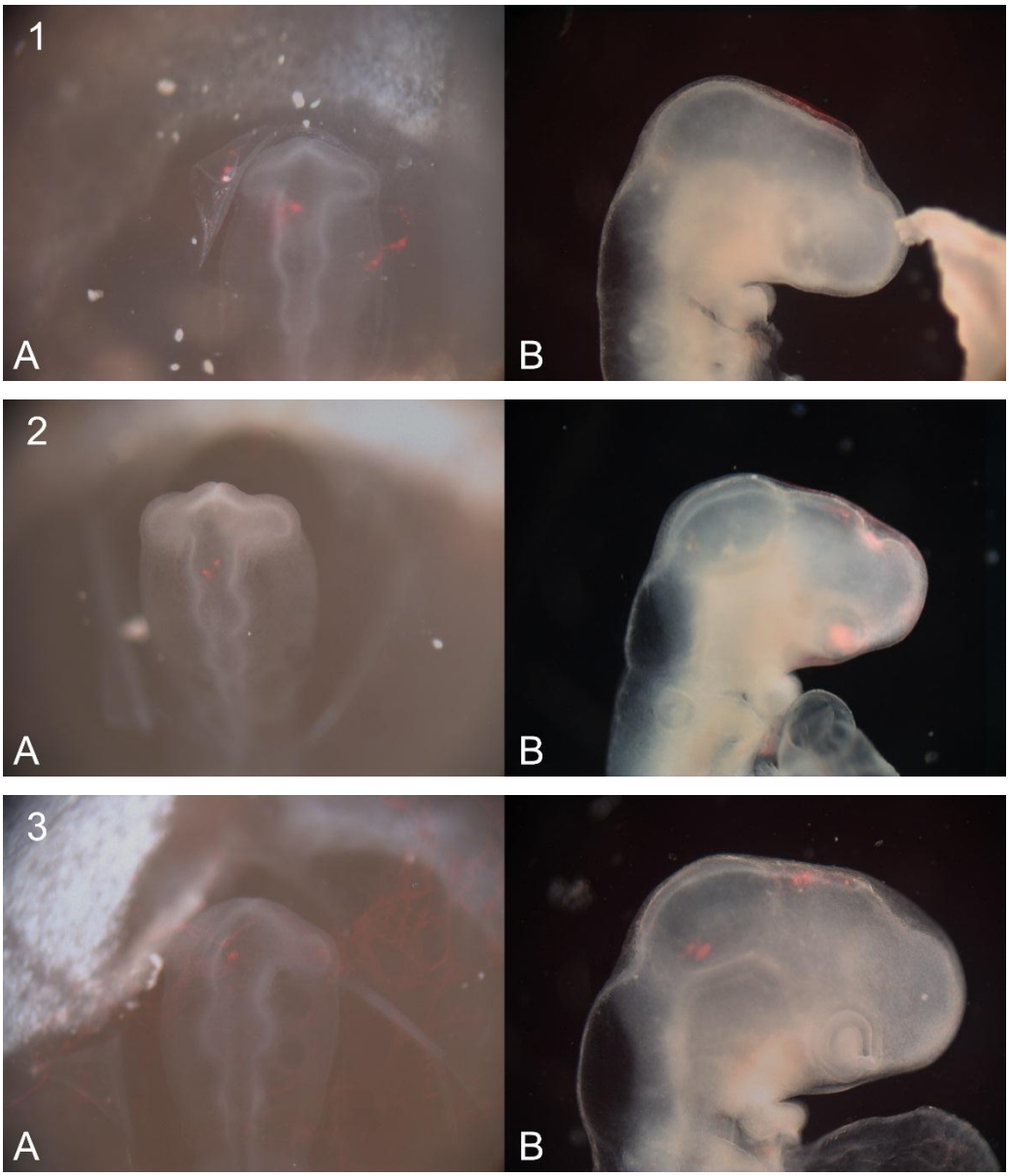

72
A
B

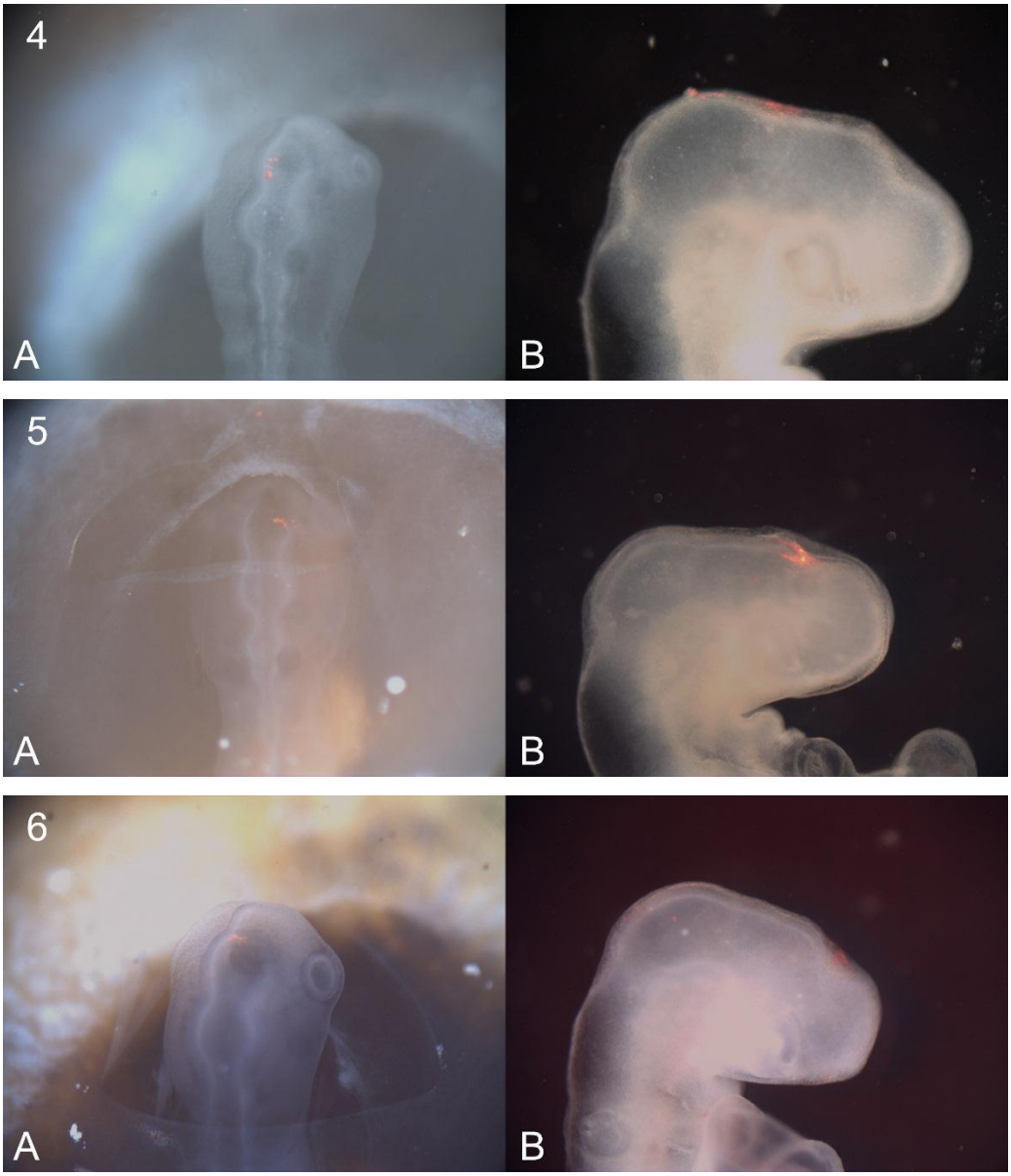

73
A
B


74
A
B

3. Preliminary Fate Mapping Data

The following pictures show DiI labels between HH10 and HH11. They are DiI labelled pictures overlapped with the raw embryo pictures. All picture A were taken before embryos were incubated (HH10-11) and all picture B were taken two days after incubation (~ HH14).




4
A
B
5
A
B
6
A
B

Acknowledgement

I would like to deeply thank Professor Claudio Stern for his supervision of my study. I would also like to thank all members in Stern lab for their help along my project, especially Dr. Claire Anderson!